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EFFECT OF LIGHT INTENSITY ON THE PHOTOSYNTHETIC EFFICIENCY OF TOMATO PLANTS[1]

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(WITH ONE FIGURE)

Introduction

The tomato stands foremost among the several vegetable plants which are cultivated as greenhouse crops. In its culture under glass, especially in the northern states, the question of sufficient light for its best development and highest productivity arises and becomes acute. The light of the natural day, during the winter months, appears to be inadequate with respect to its duration and also to its ordinary intensity.

Certainly photosynthesis is one of the most fundamental processes which condition plant behavior and production, and light is a major factor in its dynamic complex. Neglecting the characteristics of light, other than its intensity, how is its intensity related to the rate, the so-called efficiency, of photosynthesis? More particularly, what is this relationship respecting the tomato plant, when grown under greenhouse conditions? A study of this—induced by the desire to extend the knowledge disclosed by investigations already made and reported—was completed and is herein presented.

Review of literature

The effects of strong, diffused light on photosynthesis were extensively studied by MUNTZ (**10**) in 1913. He found from field observations that alfalfa produced less dry matter per square centimeter of leaf area in the summer of 1911—a summer unusually free from clouds—than in 1910, when cloudy skies prevailed much of the time. Additional observations were made in the laboratory where it was possible to equalize the amounts of water received by the lots of plants grown under different light intensities. The

[1] Journal article no. 109 n.s., Michigan Agricultural Experiment Station, East Lansing, Michigan.

results of the laboratory experiments accorded with those obtained from the work in the field. He concluded that carbon assimilation is governed and limited by the intensity of the light.

LUBIMENKO (**8**) and POPP (**15**) found that in heliophilous plants the rate of the accumulation of elaborated materials was increased with increase in the light intensity, up to an optimum point, and that any increase beyond this optimum resulted in a decrease in the rate. Heliophobous plants behaved in the same manner, the optimum, however, being at a much lower point than that for the heliophilous types.

ARTHUR, GUTHRIE, and NEWELL (**1**), working with 30 different species of plants, found the tomato to be the most sensitive to light. Light intensities of 350, 450, 760, 800, 1200 and 1400 foot-candles were used conjointly with lengths of day which ranged from 5 to 24 hours. This revealed the fact that the "time factor" was of importance. The peak of increase in carbohydrate production was reached at higher light intensities with the 12-hour day. Injurious effects resulted when the day was lengthened to 17 and 19 hours. The maximum carbohydrate increase was reached with the 17- and 19-hour day when lower light intensities were used, or at the point of injury for the higher intensities.

COMBES (**2**), working with potatoes and other tuber-forming species, found that the higher the light intensity, the greater was the accumulation of elaborated organic compounds in the storage parts of the plants. Apparently, at lower intensities the storage function ceased and the entire amount of the products of photosynthesis was consumed in the growth of the aerial parts of the plant.

DEBESTEIRO and DURAND (**3**) obtained very definite results, experimenting with the garden pea. The plant's dry-weight increase was in direct proportion to the intensity of the light employed for its irradiation.

SMITH (**18**) and YOSHII (**24**) experimented with several of the different environmental factors, and, of these several factors, light intensity had the greatest effect on the production of carbohydrates in cereals and peas. Their data show a greater production of carbohydrates under the condition of short days with bright sunlight than that of long days with reduced sunlight, although the product of the intensity and the duration of light was higher in the latter case.

KOSTYTSCHEW and KARDO-SYSSOIEWA (**7**) found that desert plants increased in carbon assimilation up to an optimum light intensity and decreased as the intensity went above this point. Later in the day, as the light intensity fell to the optimum point, the carbon assimilation again reached a maximum causing the daily curve of photosynthesis to show two peaks in its outline.

The literature which bears directly on the question of the response of the photosynthetic function to the factor of light intensity is not plentiful. The

foregoing references are not all, but are representative of those of greater importance, and, also, are sufficient to show the existence of a quantitative relationship between these two phenomena.

Besides a direct effect of light, with respect to its intensity, upon the behavior of the photosynthetic process, acting as a catalytic and energizing agent, it appears to affect certain other factors, which are essential in the process. Among these are the chlorophyll content of the leaf and its anatomical structure; the latter being important with reference to the rate of the diffusion of gases into and within the leaf's interior.

WILLSTÄTTER and STOLL (**23**) observed that the rate of photosynthesis increased with the chlorophyll content, but were unable to establish a definite quantitative relationship between the two—the function and the independent variable.

PALLADIN (**13**) and LUBIMENKO (**8**) state, on the basis of their experiments, that heliophobous plants are relatively higher in chlorophyll content than heliophilous plants. The latter investigator was able to establish the fact that the optimum light intensity for photosynthesis is lower in correspondence with reduced content of chlorophyll. Shade plants at the lower light intensities were as efficient in photosynthetic activity as non-shade plants at these same light intensities.

A number of more recent investigators, JOHNSTON (**6**), MACDOUGAL (**9**), SPOEHR (**19**), and WIESNER (**21**), working with long day plants, report that the amount of chlorophyll in the leaves of plants increased in direct proportion to the average quantity of light received by them.

SPRAGUE and SHIVE (**20**) demonstrated that there was a degree of relationship betwen the total chlorophyll content and the dry weights of tops in corn. The total quantity of chlorophyll contained in the leaves of the various strains of maize correlated closely with their dry weights at successive harvests. Strains that showed a high chlorophyll concentration per unit of leaf area also had high average rates of increase in dry weights of tops, and *vice versa*. This ratio between the total chlorophyll and dry weight of tops was practically identical with all three strains of corn tested.

EMERSON (**4**), working with *Chlorella*, observed that plant cells low in chlorophyll reached their maximum rate of photosynthesis at approximately the same light intensity as normal cells. In working with different chlorophyll concentrations in plants that were kept constant in these variations, with the same light intensity, he found that the rate of photosynthesis increased at the same speed regardless of the chlorophyll concentration. The conclusion was that chlorophyll is probably a chemical reactant in photosynthesis as well as being the photosensitizer which absorbs the radiant energy necessary in the process.

HAYDEN (5) and POOLE (14) found the spongy parenchyma cells (mesophyll) of the leaves were poorly developed in sun plants, but in shade plants these cells replaced the palisade cells.

SHIBATA (17) observed that light intensity had a definite effect on the anatomy of the leaves, in that the epidermal cells are smaller in short day plants. OSTERHOUT (12), and NIGHTINGALE and MITCHELL (11) observed that leaves were thicker and had more elongated and more densely packed palisade cells as the average light intensity was maintained at a higher point.

The literature leaves no doubt concerning the direct, and also indirect, importance of light intensity in the plant's photosynthetic behavior. The results cited from the work of ARTHUR, GUTHRIE, and NEWELL (1) are especially significant and helpful, since the tomato plant itself was among those used in their experiments. However, additional contributions from controlled experimentation are desirable and necessary before the matter of the use of artificial light in forcing houses, devoted to tomato growing and production, can be certainly and soundly determined.

Procedure

The tomato plants used in the experiment were of the Grand Rapids Forcing variety. The seeds were sown in greenhouse flats on January 26, 1933. On February 3 a large number of seedlings were selected and pricked off into two-inch pots. These were transferred on February 12 into four-inch pots and left therein until March 1, or until their development was such that they were ready for final transplantation. On that date, 36 of the plants were selected from the remaining 108, and transferred to 14–inch pots, in which they were grown singly and to full maturity.

The soil was a fairly rich orchard loam, which had been previously screened and thoroughly mixed, by having been shoveled over, in bulk. Its uniformity was as good as could be expected and secured.

The 36 plants were divided into three lots of 12 each, and each of the lots was placed, with wide spacing, on a separate greenhouse bench where the pots were surrounded by moist sand (afterwards kept moistened), to a depth of 5 inches. During the course of the experiment, the position of individual pots within each lot was systematically shifted twice each week. The number of clusters of fruit per plant was restricted to five.

A 14-hour day was maintained over the plants of each lot. Extension of the regular daylight period was accomplished by means of a 1000-watt electric lamp, with dome reflector and adjustable in height, suspended *centrally* above each group of plants. A wooden frame was constructed above each of two of the benches, under each light, and made to be vertically movable. One of these frames was covered with one layer of white cheesecloth, the other with two layers. This effected three respective intensities of the

light, both natural and artificial, for the plants: *no shade,* or *full intensity; one-half intensity, 50.4 per cent.;* and a little less than *one-fourth intensity, 22.3 per cent.* The shades were kept adjusted in their heights so as always to be approximately 24 inches above the tops of the growing plants.

It was aimed, of course, to keep the conditions of the environment, aside from the controlled variations in light intensity, the same for the three benches. Data were recorded for relative humidity, air temperature, and soil temperature, under each of the three light conditions, from March to July, by use of hygrothermographs and soil thermographs.

Additional information regarding methods—those more particularly technical—is given, where appropriate, in the following section, with its presentation of the data obtained.

Experimental results

Growth response and leaf area

Possible relationships between each of several different linear measurements of the tomato leaf and its total area were examined in a previous experiment (**16**). The length of the leaf from the base of its first leaflets to the tip of the midrib proved to be the most accurate index. The type of association was clearly curvilinear, and, specifically, parabolic in the second degree. The derived equation was $y\ (\text{area}) = 3.16 + 0.417x + 0.307x^2$.[2]

All of the leaves on each plant in each of the three lots were measured, in the manner indicated above, at intervals of three to seven days, and their areas calculated through the given equation. The data are presented in table I.

As shown by these data, expansion in leaf area was both continuously and finally the greatest for the plants under the lowest light intensity, next greatest where medium intensity prevailed, and least in the unshaded condition. The orderliness of the change in the daily rate of the increase in the foliar surface of the unshaded plants is outstanding. This rose over gradual steps to a distinct maximum (April 22), and thereafter fell off consistently to zero at the end. Differing from this, the other two maxima were reached more quickly and much earlier in the life of the plants, and were maintained over longer periods of time.

Thus, the usual result was obtained. Growth, when measured in terms of leaf area, augments under reduced light intensity. The leaves attain greater size, but commonly are thinner and may have even less total mass. The greater spread of leaf surface gives increased exposure to the light, such as it is, and tends in some degree to compensate on the whole for the lesser quantity of light received per unit of exposed surface.

[2] This leaf area equation was tested out on about 20 leaves from each of the three sets of plants and was found to apply equally well.

TABLE I

GROWTH RESPONSE IN TERMS OF LEAF AREA

DATE OF MEASUREMENT	AVERAGE LEAF AREA PER PLANT			AVERAGE DAILY INCREASE IN LEAF AREA PER PLANT		
	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
March 28	1350	1503	1625	.....	.....	.....
April 4	1649	2253	2473	42.7	107.0	116.0
April 8	1888	2687	3122	59.7	105.8	162.1
April 11	2088	2894	3594	66.6	100.4	157.1
April 15	2358	3408	4202	67.5	107.5	152.0
April 18	2668	3646	4694	102.8	77.7	158.5
April 22	3188	3901	5294	130.0	63.6	147.5
April 29	3754	4303	6259	80.7	57.3	139.0
May 6	4164	4680	6649	58.5	56.1	55.6
May 13	4325	5033	6764	23.0	48.0	16.4
May 20	4485	5433	6868	13.3	57.0	14.8
June 5	4563	5559	7072	6.5	7.9	12.7
July 3	4563	5559	7072	.....	.....	.....

GROWTH RESPONSE AND STEM ELONGATION

The measurements taken for leaf area were accompanied by determinations which gave the growth rate of the main axis of the plants, under each light treatment. The distance measured was that of the stem axis. The period of these intermittent measurements was April 4 to June 5, when the plants were pinched out at the top, and thus restricted to the production of but five fruit clusters per plant. Table II gives the data.

The responses in stem elongation were akin to those shown for increases in leaf area. Growth in height, as reflected in more attenuated internodes, was more rapid as shading was heavier, and the plants taller at the time of being topped. The maxima for the rates of elongation were in the same order as those followed by the leaves, and their occurrences in time practically identical with those which obtained for the leaves.

GROWTH RESPONSE AND FRUIT PRODUCTION

These measurements were made by tagging each fruit of each cluster, giving the date of set, and recording the number of days necessary for it to attain its full weight. This information was assembled and totaled, giving comparative figures to illustrate the effect of light intensity on fruit set and production. The data are presented in table III.

The amount of fruit set under reduced light intensity is much less than under normal light. In the early part of the season when the plants were

TABLE II

GROWTH RESPONSE IN TERMS OF STEM ELONGATION

DATE MEASURED	AVERAGE HEIGHT OF PLANT			DAILY INCREASE IN HEIGHT PER PLANT			INTERNODAL LENGTH		
	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
April 4	45	61	70	.....	.....	.....	4.8	5.5	6.2
April 8	50	68	76	1.25	1.75	1.50	4.8	5.5	6.0
April 11	54	73	87	1.33	1.66	3.66	4.8	5.6	6.2
April 15	59	82	95	1.25	2.25	2.00	4.5	5.9	5.7
April 18	64	94	100	1.66	4.00	1.66	4.7	6.7	5.9
April 22	70	96	105	2.00	0.50	1.25	5.1	6.5	6.2
April 29	74	98	112	0.57	0.28	1.00	5.2	6.4	6.5
May 6	78	100	116	0.57	0.28	0.28	5.2	5.9	6.6
May 13	81	102	117	0.42	0.28	0.14	5.4	6.0	6.2
May 20	81	102	117	0.00	0.00	0.00	5.4	6.0	6.2
June 5	81	102	117	0.00	0.00	0.00	5.4	6.0	6.2

TABLE III

GROWTH RESPONSE IN TERMS OF FRUIT PRODUCTION

Cluster no.	Total number of fruits			Total weight of fruit			Average weight of single fruits			Average days to ripen, from set		
	No shade	1 layer cheese-cloth	2 layers cheese-cloth	No shade	1 layer cheese-cloth	2 layers cheese-cloth	No shade	1 layer cheese-cloth	2 layers cheese-cloth	No shade	1 layer cheese-cloth	2 layers cheese-cloth
				gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*days*	*days*	*days*
1	57	29	0	3335	1572	0	58.5	54.2	0	48.9	53.6	0
2	75	54	20	5866	3629	1555	78.2	67.2	77.7	47.7	49.6	54.7
3	58	57	39	5277	4062	2761	90.9	71.2	70.9	47.7	49.1	53.8
4	61	47	56	5797	3896	4590	95.0	82.9	82.0	46.0	48.0	53.6
5	20	18	27	1759	1448	1913	87.9	80.4	70.8	43.1	47.2	52.0
Totals	271	205	142	22034	14607	10819	.....	.....	.....	.....	.....	.....
Av. per plant	22.5	17.0	11.8	1836.1	1217.2	901.5	.....	.....	.....	.....	.....	.....
Av. per sq. meter of leaf area				7288	3894	2229						

receiving a relatively small amount of light the fruit set was in inverse proportion to all amounts of shading, but as the season progressed and light intensity became higher the fruit set correlated closely with the foot-candle hours of light (table VII) to which the plants were exposed.

Fruit production requires a greater area of leaves in proportion to the amount of shading the plants receive. The fruits attain greater weights and ripen sooner when the light intensity is not reduced.

Growth response and total plant production

The data for total plant production determined on twelve individuals under each of the three different light intensities are shown in tables IV, V, and VI. A cursory glance at these data shows that individual tomato plants vary within wide limits. The weights taken for total plant production show a variation in plant food under each light treatment that is less than the differences in light intensity.

In accordance with expectations, increases in light available for carbohydrate formation showed a greater quantity of fresh, dry, ash, and plant food weights in the average case. The rate of photosynthesis was slowed up according to the amount of light reduction in each block. The differences in light intensity appeared to have less effect on the ash content of the plants, but are in a rather definite relation with the average results on the fresh, dry, and plant food weights in all parts of the plants. The plant food manufactured per unit of leaf area is greatest under the no-shade condition and is reduced according to the amount of shade the plants receive. This efficiency in food manufacture seems to have a definite effect on the plant material used in fruit production and is in approximately the same ratio as the average decreased plant efficiency where shaded. It would seem from this information that differences in light intensity during the seasons of the year are the direct causes for variations in plant efficiency in growth and fruit production, but plant 10 in table IV, plant 11 in table V, and plant 11 in table VI are practically equal in their efficiency under each respective condition. This appears to indicate that plant variation is responsible for some of the differences in photosynthetic activity under the different light intensities.

Environmental conditions

The experimental aim, as stated earlier, was to have the same length of day (14 hours) for the three lots of plants, while having them exposed to three different light intensities. Light measurements were made daily, at two-hour intervals, throughout the period of growth, by means of a Clements' photometer. The data for these measurements are presented in table VII.

It is clear, from examination of table VII, that the graduations of light intensity, established in the beginning by means of shading, were maintained

TABLE IV

GROWTH RESPONSE IN TERMS OF TOTAL PLANT PRODUCTION BY PLANTS NOT SHADED

PLANT	FRESH WEIGHT				DRY WEIGHT				ASH WEIGHT				PLANT FOOD WEIGHT*			
	TOPS	ROOTS	FRUIT	TOTAL	TOPS	ROOTS	FRUIT	TOTAL	TOPS	ROOTS	FRUIT	TOTAL	TOPS	ROOTS	FRUIT	TOTAL
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	629	131	2217	2977	91	22	11	226	16.4	7.9	7.9	32.2	74.6	14.1	105.1	193.8
2	382	42	1823	2247	58	8	93	159	12.2	1.6	6.5	20.3	45.8	6.4	86.5	138.7
3	409	60	1710	2179	58	7	87	152	13.9	1.4	6.0	21.3	44.1	5.6	81.0	130.7
4	574	187	2236	2997	73	28	114	215	16.8	9.8	7.9	34.5	56.2	18.2	106.1	180.5
5	604	104	3608	3316	82	15	130	227	16.4	8.0	9.1	33.5	65.6	7.0	121.3	193.9
6	523	85	1590	2198	76	12	81	169	14.5	5.4	5.6	25.5	61.5	6.6	75.4	143.5
7	382	82	1762	2226	52	12	90	154	11.4	4.1	6.3	21.8	40.6	7.9	83.7	132.2
8	476	156	1754	2386	65	26	89	180	11.7	9.6	5.9	27.2	53.3	16.4	83.1	152.8
9	529	81	1928	2538	67	12	98	177	12.2	4.8	6.8	23.8	54.8	7.2	91.2	153.2
10	464	141	1025	1630	70	26	52	148	12.4	6.2	3.6	22.2	57.6	19.8	48.4	125.8
11	507	182	1755	2444	80	33	90	203	14.8	9.9	6.3	31.0	65.2	23.1	83.7	172.0
12	608	206	1626	2440	76	23	83	182	13.8	5.7	5.8	25.3	62.2	17.3	77.2	156.7
Total	6087	1457	22034	29578	848	224	1073	2145	166.5	74.4	74.4	315.3	681.5	149.6	998.6	1829.7
Av. per plant	507	121	1836	2466	70	19	89	179	13.9	6.2	6.2	26.3	56.8	12.8	82.8	152.6
Av. per sq. meter of leaf area	2013	480	7288	9790	292	75	353	711	55	25	25	104	227	50	328	607

* Dry weight minus ash weight.

TABLE V

GROWTH RESPONSE IN TERMS OF TOTAL PLANT PRODUCTION BY PLANTS SHADED WITH ONE LAYER OF CHEESECLOTH

PLANT	FRESH WEIGHT				DRY WEIGHT				ASH WEIGHT				PLANT FOOD WEIGHT*			
	TOPS	ROOTS	FRUIT	TOTAL	TOPS	ROOTS	FRUIT	TOTAL	TOPS	ROOTS	FRUIT	TOTAL	TOPS	ROOTS	FRUIT	TOTAL
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	465	144	854	1463	62	13	45	120	11.2	4.6	2.9	18.7	50.8	8.4	42.1	101.3
2	460	187	1048	1695	65	25	55	145	14.3	11.8	3.5	29.6	50.7	13.2	51.5	115.4
3	560	116	1495	2171	66	11	78	155	13.2	2.2	4.9	20.3	52.8	8.8	73.1	134.7
4	496	140	1397	2033	56	18	73	147	12.3	6.8	4.6	23.7	43.7	11.2	68.4	123.3
5	657	213	1037	1907	77	35	54	166	16.2	17.5	3.4	37.1	60.8	17.5	50.6	128.9
6	613	280	1009	1902	63	47	53	163	12.6	19.7	3.3	35.6	50.4	27.3	49.7	127.4
7	425	269	942	1636	54	49	50	153	11.8	15.7	3.1	30.6	42.2	33.3	46.9	122.4
8	521	179	873	1573	58	23	46	127	12.7	6.4	2.9	22.0	45.3	16.6	43.1	105.0
9	554	120	1805	2479	61	17	95	173	18.3	3.4	6.0	27.7	42.7	13.6	89.0	145.3
10	458	147	1132	1737	52	20	59	131	15.6	5.0	3.7	24.3	36.4	15.0	55.3	106.7
11	563	248	1709	2520	75	38	90	203	18.7	19.8	5.7	44.2	56.3	18.2	84.3	158.8
12	502	231	1308	2041	69	38	69	176	18.6	11.4	4.3	34.3	50.4	26.6	64.7	141.7
Total	6274	2274	14609	23157	758	334	767	1959	175.5	124.3	48.3	348.1	582.5	209.7	718.7	1510.9
Av. per plant	522	189	1217	1928	63	28	64	163	14.6	10.3	4.0	29.0	48.5	17.5	59.8	125.8
Av. per sq. meter of leaf area	1670	605	3894	6169	201	89	204	499	46	33	13	93	155	56	191	406

* Dry weight minus ash weight.

TABLE VI

GROWTH RESPONSE IN TERMS OF TOTAL PLANT PRODUCTION BY PLANTS SHADED WITH TWO LAYERS OF CHEESECLOTH

Plant	Fresh weight				Dry weight				Ash weight				Plant food weight*			
	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total	Tops	Roots	Fruit	Total
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	692	155	726	1573	79	21	40	140	21.3	4.2	2.4	27.9	57.7	16.8	37.6	112.1
2	414	132	885	1431	55	16	49	120	17.6	3.2	2.9	23.7	37.4	12.8	46.1	96.3
3	492	120	983	1595	54	15	54	123	11.3	2.7	3.3	17.3	42.7	12.3	50.7	105.7
4	480	199	945	1624	68	23	52	143	19.0	5.1	3.1	27.2	49.0	17.9	48.9	115.8
5	495	77	933	1505	64	9	51	124	15.4	1.6	3.1	20.1	48.6	7.4	47.9	103.9
6	476	142	1016	1634	60	18	56	134	18.0	4.5	3.4	25.9	42.0	13.5	52.6	108.1
7	540	124	878	1542	56	13	48	117	16.8	2.3	2.9	22.0	39.2	10.7	45.1	95.0
8	582	87	510	1179	69	12	28	109	13.8	2.1	1.7	17.6	55.2	9.9	26.3	91.4
9	616	91	892	1599	78	11	49	138	17.2	2.6	2.9	22.7	60.8	8.4	46.1	115.3
10	539	32	849	1420	62	5	47	114	13.0	1.2	2.8	17.0	49.0	3.8	44.2	97.0
11	434	68	1327	1829	57	7	73	137	13.7	1.4	4.4	19.5	43.3	5.6	68.6	117.5
12	554	35	875	1464	58	6	48	112	19.1	2.7	2.9	24.7	38.9	3.3	45.1	87.3
Total	6314	1262	10819	18395	760	156	595	1511	196.2	33.6	35.8	265.6	563.8	122.4	559.2	1245.4
Av. per plant	526	105	902	1533	63	13	50	126	16.4	2.8	3.0	22.1	45.3	10.2	46.6	103.8
Av. per sq. meter of leaf area	1300	259	2230	3790	156	32	121	312	40	7	7	54	116	25	114	258

* Dry weight minus ash weight.

TABLE VII

LIGHT INTENSITY FOR PLANTS UNDER DIFFERENT TREATMENTS

MONTH	AV. DAILY LIGHT INTENSITY PER HOUR, IN APPARENT FOOT-CANDLES			TOTAL HOURS DAYLIGHT	TOTAL FOOT-CANDLE HOURS*			PERCENTAGES NO SHADE = 100		
	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
Mar. 1–30 incl.	532	251	114	434	230888	108934	49476	100	52.1	21.4
Apr. 1–30 incl.	980	497	212	420	411600	208740	89040	100	50.7	21.5
May 1–31 incl.	1133	591	246	454	514382	268314	111684	100	51.9	21.7
June 1–30 incl.	1872	973	468	459	859248	446607	214812	100	62.1	25.0
July 1– 3 incl.	1774	892	355	46	81604	41032	16330	100	50.2	20.0
Daily average during crop growth	1139.9	583.1	261.0							
Total for producing crop				1813	2,097,722	1,073,627	481,342			

* Daily average intensity × number of hours.

with close approximation, as the season advanced and ended. While the general intensity of the sunlight increased gradually for all, the three experimental conditions of full intensity, one-half intensity, and one-fourth intensity continued to hold and to be effective.

General correlation of these controlled variations in light intensity, previously shown with the differences in growth responses of the three lots of plants, is obvious. The relationship is itself negative in character for leaf area, and positive for fruit production and for total plant production. In order to facilitate inspection, certain figures from the preceding tables are brought together in table VIII.

TABLE VIII

RELATIONSHIP OF LIGHT INTENSITIES TO GROWTH RESPONSES OF THE PLANTS

LIGHT INTENSITY	TOTAL FOOT-CANDLE HOURS OF LIGHT (TABLE VI)	AV. LEAF AREA PER PLANT (TABLE I)	AVERAGE HEIGHT OF PLANT (TABLE II)	TOTAL WEIGHT OF FRUIT (TABLE III)	TOTAL FRESH WEIGHT OF PLANTS (TABLES IV, V, VI)
		sq. cm.	*cm.*	*gm.*	*gm.*
Unshaded	2097722	~~4385~~ 4563	81	22034	29578
1 layer cheesecloth	1073627	5559	102	14607	23157
2 layers cheesecloth	481342	7072	117	10819	18395

However, the association which is apparent in table VIII can have validity only in case certain other factors, which are known to condition the photosynthetic rate and, consequently, growth, remained sufficiently constant during the experimental period. Table IX gives data respecting three such factors—these being the principal ones which required consideration and control.

These three factors were relatively uniform for each block of plants. Their variations in the three treatments is regulated somewhat by the light intensity. Thus, light intensity variation mainly accounted for the plant growth responses under each condition.

DAILY PERIODS OF MEASUREMENT

The data which have been presented show, beyond doubt, a relationship between the behavior of the plants and the different light intensities under which they grew and matured. The evidence, however, is general in nature and not such as to be adequate for those mathematical processes which yield quantitative expressions of correlation.

TABLE IX

AIR TEMPERATURE, SOIL TEMPERATURE, AND RELATIVE HUMIDITY DURING GROWTH PERIOD

MONTH	AIR TEMPERATURE								
	NO SHADE			1 LAYER CHEESECLOTH			2 LAYERS CHEESECLOTH		
	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
March	73	56	62	82	58	66	80	58	67
April	88	55	65	86	55	66	86	56	66
May	86	85	66	90	55	67	90	55	66
June	98	46	73	95	48	71	95	48	71
July	106	55	76	100	54	73	102	56	70

MONTH	SOIL TEMPERATURE								
	NO SHADE			1 LAYER CHEESECLOTH			2 LAYERS CHEESECLOTH		
	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
March	62	62	62	66	61	63	72	60	64
April	63	60	62	67	60	64	70	60	66
May	63	60	62	69	60	63	74	60	66
June	65	58	62	76	59	66	82	53	74
July	67	59	63	77	60	71	82	65	74

MONTH	RELATIVE HUMIDITY								
	NO SHADE			1 LAYER CHEESECLOTH			2 LAYERS CHEESECLOTH		
	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.	MAX.	MIN.	24-HR. AV.
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
March	76	40	61	80	41	65	80	41	62
April	83	32	64	84	38	73	84	38	65
May	84	27	68	90	42	79	84	30	72
June	84	30	68	90	34	75	84	30	73
July	91	28	63	85	30	72	84	30	70

The plant materials manufactured by the plants on twelve dates, spread over the period of growth, were determined on seven periods during each of the days. This procedure for estimating the photosynthetic activity was that termed the "Modified Sachs Method." It consisted of taking 2 sq. cm. of foliage from each plant every 2 hours in sample bottles and weighing in the fresh condition. These small discs of the leaves were then heated in the oven

at 70° C. for 12 hours and then at 95° C. for 6 hours, when they were reweighed. After drying they were put in crucibles and ashed, and again reweighed. The dry weight minus the ash weight was the amount of photosynthesized product in each sample. Correction for respiration and translocation was made by adding the average loss in weight per 2 hours during the night to the difference in weight of the 2-hour samples during the day.

Light in each block of plants was measured at 2-hour intervals with a Clement's photometer in which *solio* paper is used, and comparisons made with a standard. On days when photosynthetic activity was determined, the Macbeth illuminometer was also used in order to get readings in actual number of foot-candles. The data are presented in tables X, XI, and XII.

TABLE X

PHOTOSYNTHATE AND LIGHT INTENSITIES FOR UNSHADED PLANTS

DATE	VARIABLES	PERIOD OF DAY						
		4–8 A.M.	8–10 A.M.	10–12 A.M.	12–2 P.M.	2–4 P.M.	4–6 P.M.	6–8 P.M.
April 7	Photosynthate	1.12	1.11	1.60	2.96	5.84	2.44	0.36
	Light intensity	606	1200	1411	2408	2314	1207	117
April 24	Photosynthate	1.60	1.46	0.16	2.16	4.20	1.40	1.64
	Light intensity	200	400	444	387	256	139	87
April 29	Photosynthate	1.64	3.06	3.06	4.08	1.24	2.28	1.60
	Light intensity	732	1464	2553	2553	1445	806	337
May 5	Photosynthate	1.48	1.46	1.44	1.60	1.76	0.56	0.27
	Light intensity	212	424	393	394	287	116	87
May 13	Photosynthate	1.06	1.06	1.44	2.64	0.88	0.72	0.34
	Light intensity	237	446	286	1285	2408	2408	337
May 14	Photosynthate	1.28	1.40	2.00	3.96	1.68	1.80	0.36
	Light intensity	1164	2078	2409	2503	1800	687	140
May 23	Photosynthate	2.48	1.80	0.76	1.84	7.84	1.24	1.68
	Light intensity	1206	2172	2588	2937	2730	1376	736
May 24	Photosynthate	1.52	3.20	4.96	1.32	2.92	1.76	1.60
	Light intensity	637	1230	2499	2435	2100	1350	736
June 9	Photosynthate	3.76	3.32	2.76	6.04	3.00	2.92	1.96
	Light intensity	739	1369	2533	2855	2344	964	674
June 15	Photosynthate	1.84	1.40	2.76	4.88	2.96	2.76	2.16
	Light intensity	100	186	277	278	944	910	121
June 16	Photosynthate	1.88	2.76	5.44	7.20	5.12	1.60	1.40
	Light intensity	210	330	3480	3906	3773	3039	1674
June 17	Photosynthate	4.03	2.64	1.52	9.20	5.12	1.60	1.08
	Light intensity	940	1734	1503	3155	4235	3115	1769
Av. photosynthate		582	1086	1520	2091	2053	1426	578
Light intensity		1.89	2.05	2.32	3.99	3.54	1.75	1.20

TABLE XI

PHOTOSYNTHATE AND LIGHT INTENSITIES FOR PLANTS SHADED WITH ONE LAYER OF CHEESECLOTH

DATE	VARIABLES	PERIOD OF DAY						
		4–8 A.M.	8–10 A.M.	10–12 A.M.	12–2 P.M.	2–4 P.M.	4–6 P.M.	6–8 P.M.
April 7	Photosynthate	1.00	0.76	0.16	0.28	3.44	1.44	0.36
	Light intensity	100	202	246	236	128	49	24
April 24	Photosynthate	1.00	1.06	1.36	1.28	2.16	2.80	0.80
	Light intensity	337	648	768	1245	1135	544	49
April 29	Photosynthate	0.92	1.60	1.72	1.84	1.84	2.00	0.60
	Light intensity	462	739	1182	1281	813	739	247
May 5	Photosynthate	1.42	0.88	1.60	1.04	1.60	0.12	0.16
	Light intensity	104	241	187	187	136	83	64
May 13	Photosynthate	0.80	0.60	1.80	1.32	0.52	0.24	0.28
	Light intensity	106	207	182	677	1163	1231	187
May 14	Photosynthate	1.00	1.64	1.60	1.16	1.72	0.88	0.16
	Light intensity	737	1207	1251	1409	914	352	47
May 23	Photosynthate	1.68	1.48	0.16	1.28	1.32	1.12	0.04
	Light intensity	737	894	1191	1113	1113	689	306
May 24	Photosynthate	1.68	2.24	1.48	1.04	1.52	0.96	0.40
	Light intensity	331	532	1170	1180	1259	532	306
June 9	Photosynthate	0.76	0.12	1.16	0.32	1.20	2.48	0.16
	Light intensity	312	692	1069	1424	1177	409	394
June 15	Photosynthate	0.40	1.06	1.40	2.36	1.28	2.24	0.04
	Light intensity	100	153	128	157	476	451	86
June 16	Photosynthate	1.46	2.74	0.12	2.64	2.40	1.28	0.40
	Light intensity	96	823	1793	1946	1891	1562	815
June 17	Photosynthate	4.12	2.28	2.80	4.20	4.96	2.08	0.18
	Light intensity	431	586	796	1025	2124	1515	815
Av. photosynthate		1.35	1.37	1.28	1.56	1.91	1.47	0.29
Light intensity		321	577	830	990	1024	679	279

It is shown by tables X, XI, and XII that with the unshaded plants a greater amount of light is necessary for each gram of photosynthate manufactured. Furthermore, the amounts of plant food appear to increase until 12–2 P.M. when the light intensity reaches its maximum and then decreases at a relatively similar rate with the light. Differing from this, the plants shaded show a slower increase in food manufacture relative to the light increase until the 2–4 P.M. period, when they reach the maximum, and then they decrease more rapidly in ratio with the light intensity. Greater reduction in light shows a more gradual increase in photosynthesis and there appears to be an accumulation of plant food over a longer period, or a lagging in

TABLE XII

PHOTOSYNTHATE AND LIGHT INTENSITIES FOR PLANTS SHADED WITH TWO LAYERS OF CHEESECLOTH

DATE	VARIABLES	PERIOD OF DAY						
		4–8 A.M.	8–10 A.M.	10–12 A.M.	12–2 P.M.	2–4 P.M.	4–6 P.M.	6–8 P.M.
April 7	Photosynthate	0.52	0.44	1.12	.00	2.08	1.24	0.32
	Light intensity	87	92	104	148	96	45	16
April 24	Photosynthate	0.20	0.44	0.72	1.24	2.60	2.68	0.40
	Light intensity	92	341	354	684	578	257	26
April 29	Photosynthate	0.20	1.32	1.68	1.32	1.20	1.68	0.40
	Light intensity	141	371	600	661	407	303	124
May 5	Photosynthate	0.16	0.12	0.60	1.16	1.24	1.00	0.32
	Light intensity	109	116	101	75	72	37	24
May 13	Photosynthate	0.46	0.32	0.36	0.76	0.24	0.24	0.28
	Light intensity	83	123	99	384	561	647	90
May 14	Photosynthate	0.36	0.20	0.64	0.72	0.44	0.56	0.26
	Light intensity	361	683	630	754	456	106	24
May 23	Photosynthate	0.68	1.56	0.28	1.08	2.80	1.44	0.40
	Light intensity	337	491	573	602	520	297	152
May 24	Photosynthate	1.12	0.96	1.28	0.72	2.76	1.04	0.32
	Light intensity	113	225	552	519	635	225	147
June 9	Photosynthate	0.60	0.06	0.16	0.84	0.88	1.48	0.56
	Light intensity	185	386	548	760	680	230	155
June 15	Photosynthate	0.20	0.86	0.40	3.20	1.40	0.72	0.28
	Light intensity	40	90	91	85	238	213	107
June 16	Photosynthate	0.60	0.37	0.20	3.24	1.36	1.36	0.16
	Light intensity	40	490	937	951	946	744	521
June 17	Photosynthate	4.20	2.52	1.04	1.83	4.04	1.32	0.10
	Light intensity	261	276	467	524	1080	784	521
Av.	Photosynthate	0.77	0.76	0.70	1.34	1.75	1.23	0.32
	Light intensity	154	307	421	512	522	324	158

photosynthate manufacture, when the light is decreased due to heavy shading. This appears to result in the plants exposed only to light of low intensities having a much lower basal metabolism than no-shade plants. The simple coefficients of correlation for photosynthate and light, $r = 0.5454 \pm 0.0527$, $r = 0.3012 \pm 0.0681$, and $r = 0.3034 \pm 0.0679$, demonstrate the importance of the light to the plant food manufacture under the no-shade condition as compared with shaded plants.

While temperature and humidity are similar or relatively uniform for the three lots of plants, it varied the same for each, as the day advanced. Naturally, this would be expected to be true, owing to their relationship to

light intensity and its variation. Consequently, data on temperature and humidity were taken for each of the 2-hour periods. The simple coefficients of correlation for photosynthate and temperature, $r = 0.2968 \pm 0.0681$, $r = 0.1924 \pm 0.0725$, and $r = 0.1704 \pm 0.0727$, signify that the temperature is in close relation with the light intensity and probably is intercorrelated with it. Their being lower than those for light demonstrates their slighter importance in photosynthetic activity.

The simple correlation coefficients for photosynthate and humidity, $r = -0.2099 \pm 0.0714$, $r = -0.4955 \pm 0.0565$, and $r = -0.3377 \pm 0.0663$, indicate that humidity is possibly too high for proper plant food manufacture. Their negative character signifies that the high humidity may have a tendency to hinder photosynthesis and the higher the negative correlation the greater it is reduced. This appears to be one of the contributing causes of lower plant food manufacture when the plants are shaded.

Correlation coefficients

In order to measure the direct effect of light intensity on photosynthesis, it is necessary to know how much the other environmental factors affect photosynthesis, and the relation between all of these factors. It appears that the true value of this relationship cannot be obtained directly from the raw figures, but an analysis of the data must be completed in order to determine the numerical measurements. This analysis will show the relative importance of the variation of each of these independent variables on the variation in the dependent variable and can best be demonstrated by the correlation coefficients given in table XIII.

No-shade plants.—The zero order coefficients seem to show a much greater relationship between X_2 and X_1 than between X_3 and X_1 or X_4 and X_1. The relationships between X_3 and X_1, and X_4 and X_1 are questionable because of the possibility of their being obscured by the relationships between the independent variables. Because of this we have separated the effects of the independent variable in order to get the first order coefficients. This separation tends to confirm the tentative conclusion reached with the zero order coefficients (that the major relationship is that between X_1 and X_2). The conclusions appear to be still slightly questionable because only two of the independent variables have been considered at a time. Because of this we shift to the second order coefficients. This demonstrates that when we consider the effect of variation in light intensity alone (both temperature and humidity being constant), we can explain 27.7 per cent. of the variation in photosynthate, while variation in temperature explains but 1 per cent. and variation in humidity explains but 4 per cent. of the photosynthate variation. The coefficient of multiple correlation shows that the three factors taken together explain 32.4 per cent. of the photosynthate

TABLE XIII

CORRELATION COEFFICIENTS FOR PHOTOSYNTHATE AND ENVIRONMENT*

r (0 ORDER)				r (1ST ORDER)				r (2ND ORDER)				R (MULTIPLE)			
SUB-SCRIPT	COEFFICIENT			SUB-SCRIPT	COEFFICIENT			SUB-SCRIPT	COEFFICIENT			SUB-SCRIPT	COEFFICIENT		
	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH		NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
12	0.5454 ±0.0527	0.3012 ±0.0681	0.3034 ±0.0679	12.3	0.4872 ±0.0571	0.2421 ±0.0309	0.2600 ±0.0698								
				12.4	0.5421 ±0.0528	−0.0372 ±0.0748	0.1595 ±0.0728	12.34	0.5266 ±0.0541	0.0236 ±0.0747	0.1711 ±0.0727				
13	0.2968 ±0.0681	0.1924 ±0.0721	0.1704 ±0.0727	13.2	0.0009 ±0.0749	−0.0049 ±0.0749	−0.0087 ±0.0749								
				13.4	0.2203 ±0.0716	0.01436 ±0.0733	0.0173 ±0.0748	13.24	0.1025 ±0.0741	−0.1452 ±0.0733	0.0649 ±0.0745				
14	−0.2099 ±0.0714	−0.4955 ±0.0565	−0.3377 ±0.0663	14.2	0.1716 ±0.0727	−0.4179 ±0.0618	−0.2235 ±0.0717								
				14.3	−0.0197 ±0.0748	−0.4927 ±0.0567	−0.3002 ±0.0678	14.23	0.2021 ±0.0718	−0.4457 ±0.0600	−0.2348 ±0.0708	1.234	0.5695 ±0.0505	0.5108 ±0.0553	0.3731 ±0.0644

* X_1 = photosynthate, X_2 = light intensity, X_3 = temperature, X_4 = humidity.

variation. Light intensity alone, as we have seen, accounts for 27.7 per cent. showing that temperature and humidity are negligible factors in photosynthesis except where they are correlated with light intensity.

ONE-LAYER-OF-CHEESECLOTH PLANTS.—It appears that the humidity is too high for proper use of light by the plant in photosynthesis. The humidity is consistently higher in this block of plants than in the other blocks (table IX). This may account for the reduction in photosynthate as compared with the no-shade block of plants. The correlation coefficients demonstrate this fact in every case.

TWO-LAYERS-OF-CHEESECLOTH PLANTS.—The humidity is evidently too high for the plants to utilize light to the best advantage. It appears that light intensity is possibly too low, even at the best, for proper food manufacture. The total effect of light, temperature, and humidity in this block of plants explained only 13.9 per cent. of the photosynthate variation which is about one-half that of the no-shade plants. This demonstrates that some other factors, that were not taken into consideration, probably have a definite effect on the photosynthetic activity.

SUPPLEMENTARY CONSIDERATIONS

CHLOROPHYLL CONTENT.—The relation of radiation to pigmentation is of very great importance owing to the necessity of light for the formation of pigments, and to the fact that the pigments absorb radiant energy which is essential for the photosynthetic activity of the plants. The naturally occurring plant pigments, which are found in the cell structure of the plant foliage, are chlorophyll, carotene, and xanthophyll. These pigments per unit leaf area were determined for each group of plants, at several periods during the experiment. The modified WILLSTÄTTER and STOLL method of extraction (**22**) was used, and the comparison against a standard made with the DuBoscq colorimeter. The data are given in table XIV.

It appears from table XIV that light intensity is effectual for proper chromogenesis in the tomato foliage. According to expectations, the no-shade plants contained more chlorophyll per square centimeter of leaf area, and the shaded plants showed a variation according to the amount of light the plants received. The reduction in photosynthate manufacture due to shading was relatively proportional to the amount of chlorophyll per square centimeter of leaf area. Although this reduction did not affect the chlorophyll efficiency, it did affect the total plant food manufacture.

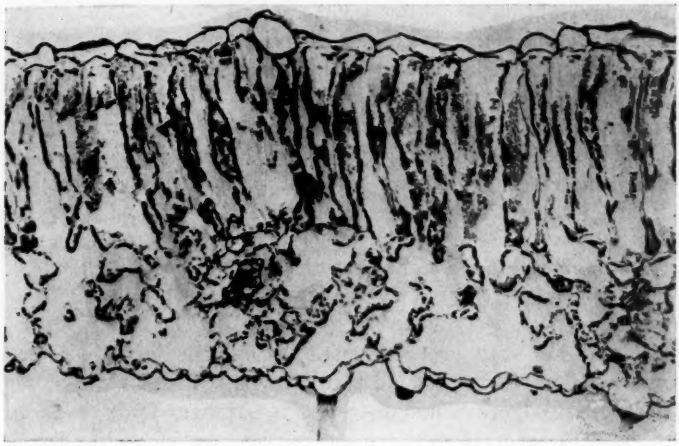
LEAF ANATOMY.—Histological sections were made, at several different times during the experimental period, of the leaves of the plants under test. Only leaves strictly comparable in position, degree of maturity, and so forth, were used. The result is given in figure 1, for the sections obtained on June 17.

TABLE XIV

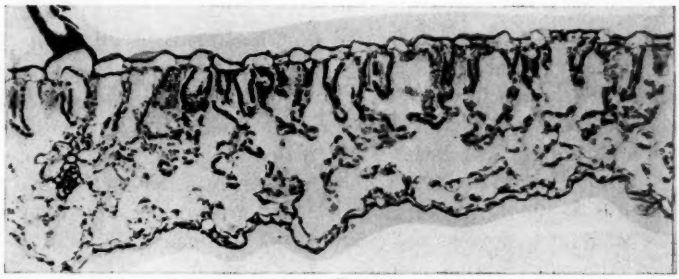
FOLIAGE PIGMENTS

	NO SHADE	1 LAYER CHEESE-CLOTH	2 LAYERS CHEESE-CLOTH
	Chlorophyll—Mg. per sq. cm.		
April 15	0.0368	0.0255	0.0189
April 15	0.0379	0.0257	0.0189
May 10	0.0477	0.0353	0.0114
May 10	0.0468	0.0377	0.0125
June 17	0.0474	0.0309	0.0110
June 17	0.0487	0.0392	0.0177
June 21	0.0401	0.0302	0.0187
June 21	0.0391	0.0300	0.0186
Total	0.3455	0.2545	0.1277
Average	0.0432	0.0318	0.0159
	Xanthophyll—Mg. per sq. cm.		
April 15	0.0024		
April 15	0.0028		
May 10	0.0020	0.0015	0.0008
May 10	0.0024	0.0014	0.0008
June 17			
June 17			
June 21	0.0028	0.0017	0.0010
June 21	0.0028	0.0014	0.0012
Total	0.0152	0.0060	0.0038
Average	0.0025	0.0015	0.0009
	Carotene—Mg. per sq. cm.		
April 15	0.0014	0.0014	0.0010
April 15	0.0017	0.0012	0.0010
May 10	0.0014	0.0011	0.0006
May 10	0.0011	0.0011	0.0006
June 17			
June 17			
June 21	0.0013	0.0011	0.0006
June 21	0.0012	0.0010	0.0006
Total	0.0081	0.0069	0.0044
Average	0.0013	0.0011	0.0007

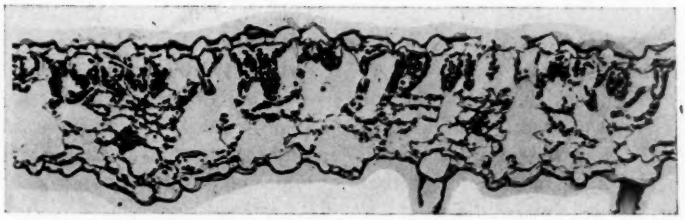
In general, the cell development of the leaves under the no-shade condition is normal, but when shaded the cells in the spongy parenchyma lack regularity in shape and are arranged loosely, so that a large part of their surface is exposed to the intercellular spaces. The greater the shade, the less the palisade parenchyma cells are developed. This demonstrates how the number of palisade layers and the density of the cell structure depends largely, either directly or indirectly, upon light intensity.



A



B



C

FIG. 1. Cross section of tomato leaves for specimens taken June 17: *A*, unshaded plant; *B*, medium shaded plant; *C*, heavily shaded plant. All × 250.

These supplementary factors are rather definitely regulated by the amount of light received by the plants, and this appears to be in order with the variation of photosynthetic activity. When the plants are exposed to

the no-shade condition the palisade cells are well-developed and their chloroplasts seem to arrange themselves so as to decrease the surface and transpiration due to the light, but when shaded they are differently arranged and thereby increase the surface for receiving light. This latter arrangement appears to increase the chloroplast's efficiency, and the greater the light reduction the more it is increased. It appears that the reduction in photosynthetic rate did not have an effect on the chloroplast efficiency. As previously stated, the light seems to have a regulatory effect on the chloroplast content and cell structure of the leaves, and this is one of the contributing causes for a decreased photosynthetic activity by the plants when shaded.[3]

Discussion

This study has dealt primarily with the influence of light intensity on photosynthetic activity of tomato plant leaves, as measured by amount of growth, fruit production, and increases in fresh and dry weights. The results show, as expected, that, on the whole, there is a close relationship between these several factors; *viz.*, with decreased light intensity there is (a) greater vegetative growth, as measured by leaf area, and both fresh and dry weight of tops and roots, (b) decreased fruit production, and (c) a decrease in the total amount of photosynthate produced by the plants. However, the increase in vegetative growth and the decreases in fruit production and total photosynthate produced are not directly proportional to the decreases in light intensity. Thus, reducing light intensity by one-half resulted in only approximately a one-fourth increase in amount of vegetative growth, a one-third decrease in fruit production, and a one-sixth decrease in total photosynthate production. Reducing light intensity to one-fourth normal resulted in only a 40 per cent. increase in vegetative growth, a one-half decrease in fruit production and a one-third decrease in total photosynthate production (table XV). This is but another way of saying that the partially shaded leaves used their limited supply of light more efficiently than the unshaded leaves used their normal supply. That is, a given quantity of light affected a greater photosynthate production in the case of the shaded plants than was true in the case of those unshaded.

Great, however, as were the differences between the growth rates, leaf areas, and fruit and photosynthate production of the several groups of plants exposed to the different light intensities, there were equally great differences between different plants within the same group, in their apparent ability to utilize their light supply for fruit and photosynthate production. Indeed, some of the individuals (no. 11) in the moderately shaded

[3] The cells of the upper part of the thick leaf in the no-shade group removes enough red and violet light rays to reduce the effectiveness on the lower leaf cells, but this blocking effect is not so apparent in the thin-shaded leaves.

TABLE XV

PLANT EFFICIENCY

	SHADING	NO. FRUITS PER PLANT	FRESH WEIGHT OF FRUIT PER PLANT	LEAF AREA PER PLANT	PLANT FOOD WEIGHT PER PLANT	LEAF AREA NECESSARY FOR PRODUCING EACH GRAM OF PLANT FOOD PER PLANT	TOTAL FOOT-CANDLE HOURS OF LIGHT RECEIVED DURING SEASON (INTENSITY × HOURS)
Table IV			*gm.*	*sq. cm.*	*gm.*	*sq. cm.*	
Plant 5	No shade	32	2608	5436	193.9	28.0	2,097,722
Plant 10	No shade	11	1025	4176	125.8	33.2	"
Average for 12 plants	No shade	22.5	1836	4563	152.6	29.2	"
Table V							
Plant 1	1 layer cheesecloth	9	854	5115	101.3	50.5	1,073,627
Plant 11	1 layer cheesecloth	22	1709	6193	158.8	38.9	"
Average for 12 plants	1 layer cheesecloth	17.0	1217	5559	125.8	44.1	"
Table VI							
Plant 11	2 layers cheesecloth	14	1327	5642	117.5	48.1	481,342
Plant 12	2 layers cheesecloth	9	875	7566	87.3	86.6	"
Average for 12 plants	2 layers cheesecloth	11.8	902	7072	103.8	68.2	"

group produced nearly as much photosynthate per unit of leaf as some of those in the unshaded group and one of those in the heavily shaded group (no. 11) produced nearly as much photosynthate per unit of leaf area as the average of those in the moderately shaded group and within 30 per cent. as much as some of the least efficient in the unshaded group (table XV).

This latter fact is of special significance for it suggests the possibility of developing a strain of plants that has a high degree of photosynthetic efficiency under conditions of low light intensity. Obviously, the producer of indoor-grown tomatoes has no control over light intensity—at least he has no practicable means of increasing it. However, if he can obtain a varietal selection which is especially adapted to the low light intensities and short days of the northern winter season, a substantial contribution will have been made to the solution of the problem of profitably growing tomatoes in the greenhouse during the winter. Little or no effort has thus far been directed toward developing such a physiological strain of tomatoes, present stocks apparently being heterozygous in this respect. The studies here reported point clearly to some of the possibilities that lie in this direction.

Summary

The effect of light intensity on the photosynthetic efficiency of tomato plants was studied by growing Grand Rapids Forcing tomato plants under three different daily average light intensities, 1139.9, 583.1, and 261.0 foot-candles. The results were as follows:

1. The responses in stem elongation and leaf-area expansion were both continuously and finally greater when the light intensity was reduced, showing a negative relationship.

2. It was indicated that when the light intensity reached a definite average the fruit would set rather freely and develop.

3. The percentages of dry matter, ash material, water, fresh weight, and elaborated food materials correlate rather closely with the light intensity received by the plants. Light intensity variation is the chief cause of differences in plant efficiency.

4. Basal plant metabolism and its contributing factors are regulated by the amount of light received by the plants.

5. The increase in the multiple correlations (when the elaborated food materials are the dependent variable, and light intensity, humidity, and temperature are the independent variables) over the simple correlations under each degree of light intensity is evidence that there is interrelation between factors regulating the plant food manufacture. The coefficients of determination demonstrate that light intensity alone accounts for 32.4 per cent. of the photosynthate variation and that temperature and humidity are negligible factors only when correlated with light intensity—humidity becom-

ing a critical factor in photosynthesis when the light intensity is reduced.

6. The light intensity appeared to have a regulatory effect on the average amounts of chlorophyll per square meter of leaf area. The chloroplasts in the leaves arranged themselves so as to get the greatest amount of light when it was reduced.

7. The leaf anatomy shows abnormal cell development when the plants are shaded. This abnormality consists of loosely arranged, irregular spongy parenchyma cells and a reduction in size, density, and number of palisade cells.

8. It is evident that light intensity averaging 1139.9 foot-candles daily during the growth of the tomato plants had a greater effect in promoting chlorophyll formation, fruit production, and photosynthetic efficiency than light of a daily average of 583.1 foot-candles, and this, in turn, had a similar greater effect than that on the plants receiving a daily average light intensity of 261.0 foot-candles.

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EFFECT OF CERTAIN NUTRIENT DEFICIENCIES ON STOMATAL BEHAVIOR

MANHER C. DESAI

(WITH TEN FIGURES)

Introduction

Although a great deal of research has been done on stomata, nevertheless, the facts regarding these important plant structures are still fragmentary and there are differences of opinion as to their fundamental characteristics. In 1895 BLACKMAN (**3**) demonstrated the importance of stomata in gaseous exchange, although the possible relations of stomata to transpiration had long been recognized. Regarding the regulatory rôle of stomata in respect to transpiration, differences of opinion exist. That the stomata open and close periodically during twenty-four hours has been established. Various theories have been advanced concerning the mechanism of opening and closure and the cellular changes associated with these. According to some, the changes of starch to sugar and *vice versa* with consequent changes in osmotic relations are responsible for the opening and closure. SCARTH (**31**), in recent years, has emphasized the importance of changes in the pH of the guard-cell vacuome due to changes in carbon dioxide content of the leaf, the opening and closure of the stomata being dependent directly or otherwise upon the effects of pH on starch-sugar changes or protein hydration, or both.

Whatever eventually may be found to be the actual mechanism involved in the opening and closure of stomata, it is clear that the metabolism of the guard cells and the adjacent tissues must also be involved. While much attention has been given to the direct influence of light, of leaf water content, and of chemical reagents and reactions on stomatal behavior, no attention, except for the work of PLEASANTS (**27**), has been given to the part played by nutrients on stomatal behavior. There has been some work reported on the effect of nutrient deficiency on the rate of photosynthesis and respiration by GRUZIT and HIBBARD (**11**), BRIGGS (**4**), LYON (**20, 21**), GREGORY and RICHARDS (**8**), and others.

Since stomatal behavior through its effects on the loss of water and gaseous exchange may markedly influence plant behavior, a fuller knowledge of the effect of various factors on stomatal behavior is desirable. Of the factors that are of importance, theoretically and practically, that of mineral nutrient supply may be of considerable significance.

Materials and methods

Material

In order to find whether stomatal behavior is influenced by mineral nutrition, various types of plants were grown in culture media deficient in potassium, phosphorus, or nitrogen. Some of the plants included in the experiments were maize (*Zea mays*), *Tradescantia* sp., Canada field peas (*Pisum sativum*), red kidney beans (*Phaseolus vulgaris*), and tobacco (*Nicotiana tabacum*).

These plants represent two distinct structural types of stomata found among the angiosperms, (1) the grass type and (2) the ordinary type. The ordinary type can be subdivided, on the basis of arrangement, into two classes: (a) regular, and (b) scattered.

The stomata of the regular type show a tendency towards formation of rows with the long axis of each stoma practically parallel to the long axis of the leaf, while the accessory cells, which are often present, are like other epidermal cells, except that they are generally a little smaller. The scattered stomata have no tendency towards formation of rows and there is no plan in the orientation of their axes, which lie at any angle to the long axis of the leaf. Many have no definite accessory cells. *Tradescantia,* some of the orchids (Orchidaceae), and certain other plants have the regular type, while *Pisum, Phaseolus, Nicotiana,* and many other dicotyledonous plants have the scattered type.

In all the experiments care has been taken to use functioning leaves. The first two leaves near the growing point are decidedly immature, while those near the basal end of the shoot are quite old, as shown by their yellowing and dying. In these cases, the stomatal behavior will not be normal. On the other hand, the third and fourth leaves have been found to be active and mature. The growth curve for the leaf tends to flatten when the leaf comes to be the third or fourth from the tip of the stem. Dr. Parris (oral communication) has found that at this stage the assimilation rate of *Phaseolus* reaches a maximum. These leaves, being neither too young nor too old, were used for the work reported here.

With *Phaseolus,* two opposite leaflets of a compound leaf were used, while in *Pisum* the first pairs of leaflets, counting from the stipules, were used. At least two plants were taken for each observation. These were so placed that the third or fourth leaf of each plant had a uniform light exposure.

At the time of observation, the readings were taken from near the tip of the leaf. In *Zea, Tradescantia* sp., and in most monocotyledonous plants, the first part of the leaf to emerge from the sheath is the tip, the base being the last. In these cases the use of the leaf tip prevents any accidental use of immature stomata.

With the dicotyledonous plants used, observations showed that the region of the tip generally stops growth first, the basal region continuing growth the longest. The work by AVERY (**2**) and others on tobacco shows this to be true.

Preliminary experiments with *Pisum* and other plants showed that the stomata from the tip region are much more sensitive to slight changes in light, leaf water content, and other environmental factors. This is especially true in plants with large leaves.

METHODS OF STOMATAL STUDY

One method for the study of stomatal behavior is rarely suited to all plants. The several methods available may be grouped as (1) direct and (2) indirect.

At the beginning of this work, it was decided to test all the available methods and to adopt the most suitable ones. MAXIMOV (**23**) has discussed some of the features of these methods in detail, and his work should be consulted for some of the features omitted.

Three indirect methods in general use for the study of stomatal behavior are (a) the MOLISCH injection method, (b) the porometer method, and (c) the hygrometric methods. By the MOLISCH method a qualitative reading can be obtained. The method, though quite useful and rapid under field conditions, could not be used to advantage in comparative studies where stomatal measurements differ often by not more than a micron or so. Various investigators have tried to make it a quantitative method. My own attempts to do so have so far given no satisfactory results.

The porometer method, although having certain disadvantages, has distinct advantages of its own when used with care and discretion. The important sources of error have been studied in detail by KNIGHT (**13**). This method has at least two disadvantages in a critical study. It measures resistance to a pressure flow, which is different from a resistance to diffusion, which is the normal method of gas movement. If what ASHBY (**1**) found is true, it also shows a flow of 15 to 26 per cent. when the pores *appear* to be completely closed. Furthermore, ASHBY's pressure flow measurements were subject to temperature changes, and he makes no attempt to correct for this when comparing with pore size.

The most important use for this method is to get correlative data on four distinct plant processes every hour, or possibly oftener. These processes are stomatal behavior, transpiration, carbon dioxide assimilation, and respiration. No use of this method has been made for such purposes except by MASKELL (**22**), who measured the carbon dioxide assimilation and stomatal opening at the same time. Nobody has used this method to get correlative data on all four of these processes.

Because of the lack of sensitiveness of this method, when the stomata are mostly closed, and also because the changes in the size of intercellular spaces offer varying resistances, this method was not suitable for the present work.

The hygrometric methods got impetus from F. DARWIN (*cf.*, MAXIMOV's monograph), who assumed that transpiration was proportional to the stomatal opening. When it was shown that such an assumption was not strictly true, the hygrometric methods came into general disuse except occasionally by some authors in measuring stomatal opening.

The best hygrometric method is the cobalt chloride paper method. It has been used extensively in measurement of transpiration. Here the data for transpiration do not give absolute transpiration but transpiration under certain standard conditions. The application of cobalt chloride paper, as is done in the most improved form of this method, causes all the water vapor issuing from the leaf to be absorbed rather quickly. The result is that the gradient of diffusion of water vapor becomes much steeper than it was before the application of the cobalt chloride paper. This diffusion gradient will remain approximately constant, except when the leaf is near wilting or just before the color change takes place. Here the rate of movement of water vapor will be conditioned only by the stomatal aperture if we assume that the humidity in the substomatal chambers remains constant and if the cuticular transpiration is not great. Then the speed of color change will denote roughly the size of the stomatal aperture.

This method and the porometer method have a certain resemblance, since each measures the movement of gas. The cobalt chloride method differs from the porometer method in that it measures the diffusion of water vapor under standard conditions instead of the flow of gases under diminished pressure. But the varying resistance offered by the intercellular spaces may play a rôle in limiting the usefulness of this as well as the porometer method. If the intercellular spaces at certain times offer greater resistance to diffusion of water vapor than does the stomatal aperture we may not measure the aperture at that particular time but the rate of diffusion conditioned by the intercellular spaces. How important a rôle the intercellular resistance plays in conditioning the diffusion of gases in and out of a leaf is not very well known and needs to be studied.

In the cobalt chloride method there is an additional source of error. During the time of observation carbon dioxide movement is hindered and the cobalt chloride paper prevents light from reaching the covered part of the leaf. As the time of such covering will vary greatly depending upon the rate of diffusion of water vapor from the leaf, another variable is introduced as a source of error.

The cobalt chloride method is probably the best among the indirect methods and has the advantage of quickness and practicality in field work over

any other method of stomatal study. It was not used in the present investigation, however, as the strip method, in many ways, seemed more reliable.

The three direct methods for studying stomatal behavior are (a) the LONG and CLEMENTS' collodion method, (b) the direct visual method, and (c) LLOYD's strip method.

In 1934, LONG and CLEMENTS (**17**) published their paper on the collodion method, discussing the method in detail. Apparently, the advantage of this method was that the same leaf could be used over and over again. But it was noticed early in my own trials that the leaves of *Tradescantia* often cooled more than 15° C., after the collodion was applied. It is doubtful whether this method does away with this disadvantage of LLOYD's strip method. When an attempt was made to reduce this cooling effect by removing the strip earlier, there was great distortion in the direction of the pull.

Upon exactly duplicating the technique of the above authors, it was found that the shrinkage caused by evaporation of the solvents was not 3 to 4 per cent., as LONG and CLEMENTS claim, but about 10 per cent. This error could be ascribed to the method of getting the shrinkage data. They coated an eyepiece micrometer with collodion. After the film was dry it was stripped off. The reading on the strip was compared to the actual reading on the micrometer. They found that the shrinkage was about 3 to 4 per cent., which was correct. The micrometer, however. is a stiff object as compared with the usual types of leaves. Moreover, the collodion adheres more firmly to dry glass than to a leaf. The leaves of *Tradescantia* sp. are somewhat fleshy, but not thick. When the collodion membrane on such a leaf dries, a marked tension develops, often causing the leaf to curl. Shrinkage obtained in strips of collodion taken from a leaf is not the same as that of collodion strips taken from a glass micrometer.

The collodion method was not used because of these errors and because of the difficulty of getting exact measurements of the opening. It has almost no advantage over LLOYD's strip method except in cases where the latter could not be used, *e.g.*, where the material is comparatively stout, as in pods of *Phaseolus*. Unless a non-shrinking, rapidly drying material is found to replace the ordinary collodion, this method must be relegated to a secondary place.

Direct observation of stomata *in situ* under a microscope is considered by most workers to be the most reliable. This method can be used to advantage in plants with comparatively thin leaves and large stomata. Moreover, many of the plants, such as *Tradescantia* sp., are very difficult to strip when turgid. This plant, with its large stomata and small leaves, lends itself nicely to this method of stomatal investigation.

LLOYD (**15**) used a microscope with a small flask containing water as a condenser and heat absorber. LOFTFIELD (**16**) arranged a microscope stand

for direct observation but unfortunately neglected to describe his technique. Various individuals have described techniques for direct observation of stomata, but, as a whole, they are more suitable for counting the stomata per unit area of leaf than for measuring their aperture.

For the present investigation an apparatus was set up to study the stomata *in situ*. The accompanying diagram (fig. 1) shows some of the features of the apparatus. There are two strong vertical rods (2) about 1.5 inches diameter on which are fixed the horizontal rods (1 & 3) about 0.75 inches in diameter. The horizontal rod (1) is so fixed that by loosening the screw (8) the microscope can be slid along the rod (1) and can be taken to other plants mounted in the same manner as the flask (4) along the rod (3). The glass-slide stage (6), with leaf in position (drawn diagrammatically), is shown in operating position on the microscope. In the right corner of the illustration the glass-slide stage is drawn showing details of construction, *a* representing material made of slightly stiff celluloid. The mirror (9) was swung out of position in the daytime while it was used to reflect light from a source not shown in the illustration. A small wooden piece (7) is tied to each of the vertical rods, only one of which is shown at (2). To these two wooden rods (7) was tied a soft string which supported the plants. At (5) is seen the old style microscope stand from which the base is removed. It is held in position by ordinary laboratory clamps. Objective lenses used were 4-mm. and 8-mm. Zeiss achromatic objectives with a 15× ocular. The green color of the leaves served more or less as a color filter.

The condenser was eliminated from the apparatus and natural light was used during the daylight hours. At night or on a dull day, light from a 60-watt frosted Mazda electric lamp was reflected from the microscope mirror through the leaf to the microscope objective. It has been found that light from a 500-watt bulb with a bright reflector five feet from the leaf and an 8-inch electric fan four feet from the microscope caused a slight opening of the stomata in nine minutes, while easily visible opening took place in thirteen minutes. It was concluded from such experiments and others that the comparatively weak light from a 60-watt lamp at a distance of 5 feet for a maximum period of two minutes did not change the aperture to a noticeable degree.

Ordinary stages on microscopes are not only too large but they exclude light from the leaf during the period of observation. Moreover, with *Tradescantia* a slight carelessness in handling causes breaking at the growing point, making the specimen useless for further observation. For these reasons, the ordinary microscope stage was replaced by a glass slide.

LOFTFIELD put the leaf between a glass slide and a coverslip. This he clamped very lightly. This method not only causes slight pressure but partially prevents efficacious gas exchange. This was thought undesirable.

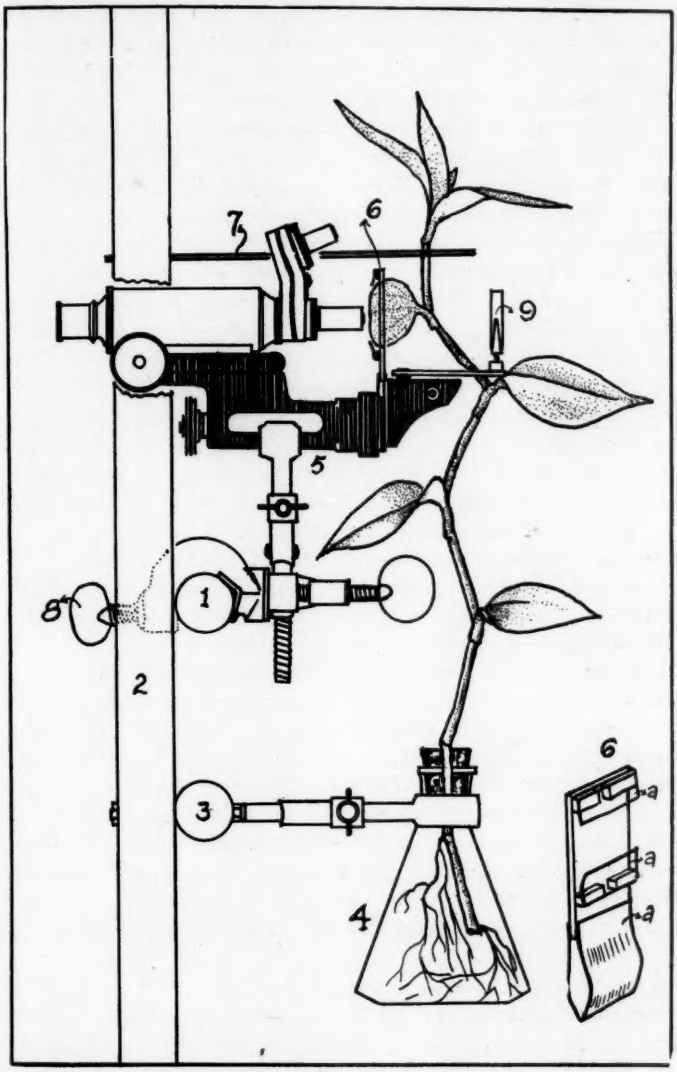

FIG. 1. Apparatus for direct visual observation of stomata *in situ*.

Two pieces of celluloid were mounted on a glass slide in such a way that a leaf could be slipped into position quickly and without undue disturbance. The illustration, figure 1(6), clearly shows this method. This arrange-

ment not only keeps the stomata from coming in contact with the glass slide (as this plant has no stomata on the upper side of its leaves), but also keeps the leaf flat so that all of the field is in focus.

With this apparatus the microscope can be slid from plant to plant without any trouble and a leaf can be brought under the field of the microscope with the least possible disturbance and change in the position of the leaf. The leaves of the plants were always left in their natural position and the microscope tilted as needed to get the leaf on the slide.

For observation with this method, two plants per treatment were selected and their leaves kept in as similar a position as possible with reference to light and convenience for microscopic observation. At the proper time the leaf was brought carefully into the field and 15 stomata rapidly measured.

This method is excellent when working with only one treatment. But when a comparative study has to be made of four treatments, as was the case in the present investigation, the method is rather a tax on the worker. LOFTFIELD did not measure more than ten stomata, but in the present work measuring 120 stomata per hour was very difficult. Because of this, no attempt was made to make very accurate measurements of stomatal apertures, as was done in the case of the strip method. The observations obtained by this method agreed essentially with those obtained by the strip method and hence the data reported are only for the strip method.

In the present case, direct observation *in situ* was tried not only with transmitted light but also with reflected light. The apparatus used was Leitz Ultropak. This microscope, though suited for stomatal counts, has not proved satisfactory for measurement of stomatal apertures. One of the reasons is that there is a large refraction from different parts of the walls of the guard cells making up the stomatal aperture. The situation was but slightly improved by the use of color filters, and other devices. Even if this difficulty was overcome, the microscope became useless in bright daylight in the greenhouse. Use of hoods over the head of the observer and ocular helped a little but the whole outfit became unwieldy in the small space of a greenhouse.

LLOYD (**14**) first reported on his strip method in 1908. It was put on a firmer basis by LOFTFIELD (**16**). Since that time various individuals have utilized this method. The strip method has been found to be as exact as the direct visual method if the precautions mentioned by LOFTFIELD are strictly adhered to and if observations are made on material mounted in absolute alcohol instead of the original mounting in balsam. But the instruments employed by LOFTFIELD were too cumbersome to manipulate without much practice. It has been found that a sharp, pointed scalpel in conjunction with the thumb of the right hand is all that is necessary for speedy stripping. ERNEST (**5**) has used a similar technique in her study of suction-

tension gradients. With this simplification it should not take more than one second to get a strip and plunge it into a vial of absolute alcohol.

Many plants have leaves which cannot be stripped easily. Plants like *Zea mays* and *Phaseolus* do not yield good strips when deficient in phosphorus or nitrogen. Here the veins are a little closer together, causing the strips to break easily. Some plants, like *Pyrus malus* and others, have leaves distinctly divided into small islets by the close, coarse venation. In this case the leaves are often quite brittle when folded between the fingers. Such leaves seldom give good strips, while plants with more pliable leaves very often can be stripped easily.

Moreover, some leaves (*e.g., Pisum* and *Impatiens* sp.) can be stripped much more easily when the strip is taken in a region where the distance between two major lateral veins is appreciable, and where minor anastomosing veins are not too prominent. In such a case, if the strip is begun nearer the midvein and carried out towards the margins, a long piece of epidermis can be obtained with ease. In the grasses and in all leaves with parallel venation, the strip could be profitably started between two large parallel veins and pulled out towards the tip. From *Zea mays* the writer has been able to obtain strips as long as 1 cm. and a little less than 5 mm. wide with a very small amount of tissue from the palisade region. A whole leaf of *Pisum* can be stripped in one piece with little difficulty.

While investigating these methods, a simple test of so-called "strippability" of leaves was accidentally hit upon. It was found that if a leaf from a plant gives an epidermal strip easily when peeled by means of the nails on the thumb and second finger, the plant may be taken as good material for stripping. This method should be tried when the leaves are turgid, since in many plants the leaves strip rather easily when slightly wilted but not when turgid (*e.g., Tradescantia* sp.).

A troublesome feature of the strip method is the large number of vials needed when stomatal behavior in variously treated plants is being studied. To eliminate this difficulty, bags were made from "onion tissue paper." LePage's liquid glue was used because it is practically insoluble in absolute alcohol. Small paper bags (6.5×3.5 cm.) were prepared and kept in absolute alcohol. After about twenty-four hours this alcohol was replaced with fresh alcohol. The strips were plunged into vials (about $\frac{1}{3}$ full) containing absolute alcohol, left there for about fifteen minutes, and occasionally shaken to prevent dilution of alcohol around the strip. The shaking of vials is quite important as a small amount of leaf tissue often accompanies the strip. After this the strips were transferred to the bags and left there till needed. Generally the bags were marked in pencil with information about the strip before they were transferred. The paper bags were stored in well-stoppered wide-mouthed bottles containing absolute alcohol.

Whenever required for making the measurements, the strips were removed from the paper bags, trimmed, mounted on a clean, dry glass slide and covered with a no. 1 square coverslip. *The mounting medium was absolute alcohol.* The coverslip was sealed to the glass slide by means of ordinary beeswax. In no stage of this processing were the strips allowed to become dry.

Early in the work it was noted that mounting the strips in Canada balsam (with xylol as solvent) brought many post-mortem changes in the stomatal aperture. This was noted very often in *Zea mays*. For this reason the usual technique was abandoned and the material was mounted in absolute alcohol. This not only does away with the most important defect of LLOYD's strip method but also saves an enormous amount of time in the processing. Observations regarding the post-mortem changes in stomatal postures have been amply corroborated by the work of NADEL (**25**). The writer agrees with OPPENHEIMER (**26**) in that much could be gained from the study of individual stomata, but he does not think that conclusions based on the study of a population of stomata are very far removed from those arrived at by the study of an individual stoma. The criticism of OPPENHEIMER (**26**) of the earlier work on stomata using the strip method is valid, but is not pertinent to the work reported here.

In this work it was noted that staining is not necessary with the ordinary type of stomata, but it is with stomata of the grass type. In order to obtain uniform results with these, with a minimum expenditure of time, all the bags containing the strips were placed in the staining jar at the same time. After the right stage of staining was attained, the bags were cleared twice in absolute alcohol. The strips were then removed, trimmed, and mounted as usual.

In the case of stomata of the ordinary type, at least thirty were measured under a 1.8-mm. oil immersion objective and a 10× Bausch and Lomb filar micrometer. These were taken from 2 or 3 strips obtained from at least two plants. This insures much more accurate measurements of stomatal apertures than could be obtained with ordinary ocular micrometers. In the case of the grass type, a measurement of about 60 to 100 stomata per treatment per reading was the rule. This largely eliminated the error of measuring a small number of stomata. Thus the objection raised against the strip method by ASHBY (**1**) and others is not pertinent here. Even by measuring fewer stomata, ASHBY has found that the stomatal curves, obtained by the strip method and the porometer method, run close together, except where the stomata are nearly closed. The error here probably lies with the porometer method.

KNIGHT (**13**) points out that the weakness of the strip method is the violent treatment accorded to the leaf. Some think that holding a leaf be-

tween two fingers momentarily (as is done while stripping) is a violent treatment, but the observations of SCARTH, WHYTE, and BROWN (**32**) do not corroborate it. SCARTH (**31**) observed that "mechanical stimulation of the plant or leaves by shaking, rubbing, exposing to blasts of air, etc., which was found by KNIGHT to induce partial and temporary closure in certain plants, seems to have no effect on *Zebrina pendula* or (so far as observed) on any species" with which he worked. My experience agrees essentially with that of SCARTH. If cutting a leaf, as is done in stripping, is a violent treatment then this difficulty can be overcome by not using the same leaf twice. This was the practice in the present investigation, although the observations of SCARTH *et al.* (**32**) indicate that the injurious effect does not extend beyond a millimeter or so from the actual wound. When he allowed the "shaved off" epidermis to be immersed in water, visible closure began within a few minutes and became practically complete in about fifteen minutes. The stripping in the present method is so rapid (not taking more than a second or so) that there is very little chance for the stomatal aperture to change enough to be measured by a Filar micrometer. "Stripping" of the epidermis, as is done in the present work, has been found to be better and speedier than the "shaving" or the "slicing" technique of various workers. This is especially true when stomatal apertures are studied in fixed preparations.

As a quantitative method, the strip method, if carefully used (with the modifications reported here), seems to be the best for the study of stomatal behavior. It is superior to the direct visual method in that there is less strain on the worker's eyes. It gives as accurate data as the direct visual method and, for a longer period of observation, it is probably even more accurate because there is no fatigue. It allows rechecking of observations. Another important advantage of the strip method is that stomatal behavior observed during a period of 12 or 24 hours is not that of one or two plants only but of a population of plants; hence it reveals more accurately the probable behavior in plants than the porometer or the direct visual methods. But the advantage of the porometer method is evident when the gaseous exchange through one particular set of stomata is to be measured. All the methods except the porometer and the direct visual method entail the growing of a large number of plants.

CULTURAL METHODS

The cultural methods used during the course of the present investigation were water, sand, and soil cultures. The minimum number of replications per treatment was 20, the maximum, 50. In water and sand cultures a 1 to 10 dilution of modified Knop's solution was used, while in soil cultures the following solution was applied to each pot containing an average of 900 gm. of sandy loam soil:

1 gm.	KH_2PO_4
1 "	NH_4NO_3
20 mg.	ferric tartrate
1 liter	distilled water

This was applied in small amounts at intervals during the entire growing season of a series. Whenever a certain nutrient element was omitted from the standard culture solution it was replaced by equivalent amounts of some other commonly used element, such as sodium for potassium.

The present investigation was carried out during 1933, 1934, and part of 1935. Most of the experiments were carried out in the greenhouses at Cornell University. In each series the data were collected under a standard condition of air movement, *i.e.*, three 8-inch fans were kept continuously running under the greenhouse bench on the days when the data were obtained.

The purpose of the present work was to study the effect of deficiency and not the effect of total lack of these nutrients. No attempt was made to use refined cultural methods such as purifying the salts, etc. It was most important to have the plant make fair growth in spite of the deficiency. For this reason soil seemed the best medium in which to grow the plants. Here the plants generally showed clear-cut deficiency symptoms which could be checked by the recovery that took place when the deficient nutrient was added. In obtaining each series of data it was customary to see whether the symptoms noted at the time of taking the readings were due to deficiency of the nutrient not provided, or to deficiency of another nutrient. Some of the data where such complications prevailed were discarded.

Experimental results

A. Deficiency symptoms

According to the experience of McMurtrey (**24**), Karraker and Bortner (**12**), Reed and Haas (**29**), Jacob (**11**), and others, different nutrient deficiences cause definite symptoms. These could be used more conveniently as criteria for nutrient deficiencies than cumbersome and uncertain analyses especially in controlled experiments. The deficiency symptoms observed here are, in the main, similar to those noted by other investigators.

In the full-nutrient cultures the plants were healthy, having a uniform green color in all of the leaves (figs. 2, 3). Here the older leaves continued to remain green for a longer period of time than in any deficient cultures. The dry weights were also generally higher, with the top-root ratio rather large. The roots were not longer than in the deficient cultures but were abundantly branched.

The plants grown in culture media deficient in potassium were generally smaller in size (fig. 10) than those grown in full nutrient cultures. The

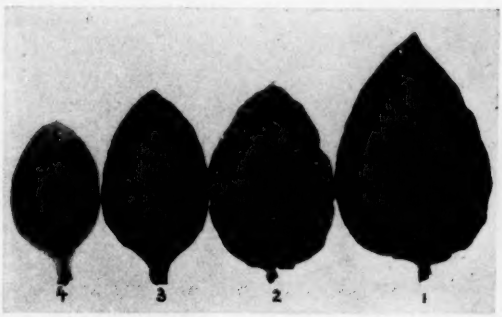

FIG. 2. Symptoms of nutrient deficiency in leaves of *Nicotiana* (series TO2). Leaves were obtained from the basal region of plants shown in figure 10.

1. Supplied with full-nutrient solution
2. Deficient in potassium
3. Deficient in phosphorus
4. Deficient in nitrogen

margins and tips of the older leaves at first turned yellow and then showed brownish necrotic areas in the mesophyll tissue between the veins, but that close to the veins, as a rule, remained dark green (figs. 2, 3). Finally, the older leaves dried up, beginning with the tip and margins, and often remained hanging on the stem in a shriveled condition. As compared with the older leaves, the younger ones remained dark green with a bluish cast. The younger leaves did not show yellowing nor necrotic areas.

In contradiction to JACOB (**11**), no "falling over due to withering of the stem at the base" was noted in plants deficient in potassium. Also no accumulation of purplish or reddish pigment in veins was observed, although such pigmentation was noted on the margins and midrib in *Zea, Raphanus,* and other plants when deficient in nitrogen.

Pisum did not show any definite symptoms of potassium deficiency, except that they were slightly smaller and their older leaves dried earlier. Symptoms of potassium deficiency of the ordinary type have been noted in sweet peas (*Lathyrus odorata*). The top-root ratio in plants suffering from potassium deficiency was not as large as in those grown in full nutrient cultures (table III).

When not supplied with phosphorus, the leaves of the plants developed a dark green color with a dusky or brownish tinge. The leaf size was greatly reduced (fig. 2). *Nicotiana* plants have been observed to show development of ochre brown spots (fig. 3), especially in the older leaves. This condition has appeared consistently in all of the experiments with *Nicotiana* and does not seem to be due to chance. A similar type of spotting was noted by KARRAKER and BORTNER (**12**), and MCMURTREY (**24**), but the latter author

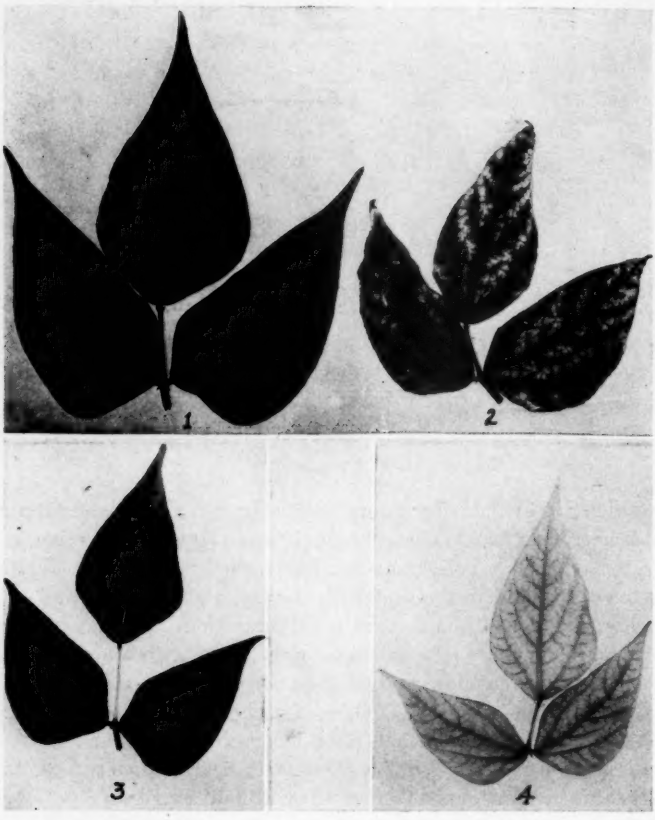

FIG. 3. Symptoms of nutrient deficiency in leaves of *Phaseolus* (series B1). Leaves were obtained from the basal region of the plants, where deficiency symptoms are markedly shown. Stomatal behavior in this series is shown in figure 6.

1. Supplied with full-nutrient solution
2. Deficient in potassium
3. Deficient in phosphorus
4. Deficient in nitrogen

is not sure that this spotting was due to phosphorus deficiency. In the present work on *Nicotiana,* the severity of this spotting decreased when the plants were supplied with phosphorus. No spotting of this type has been observed in *Phaseolus* or other plants used. This contradicts the observation of JACOB (**11**), who noted spotting in *Phaseolus* deficient in phosphorus. However, this may be a matter of relative deficiency.

In the case of *Tradescantia,* the leaves became mostly small, with the similar brownish deep green color and a deep purplish tinge over the lower

side. The leaves became fleshy and very brittle when folded, which is in agreement with the observation of PRIDHAM (**28**). In all cases, the phosphorus deficiency caused rapid drying of the lower leaves. The dried leaves generally showed a dark brown color. *Pisum* again showed no definite symptoms, corroborating JACOB (**11**). The internodes were longer in *Tradescantia, Phaseolus,* and *Zea,* and the plants were spindling. In practically all cases recovery took place when the plants were supplied with phosphorus in the form of phosphate.

In the plants deficient in nitrogen, there was a definite restriction of new growth, as in the phosphorus-deficient plants. The internodes, on the contrary, were short, giving the plants a rosette-like appearance. The leaves were smaller, thicker, and more brittle in *Tradescantia,* and showed purplish margins. A slight pink coloration was also noted on the lower side of the leaves of this species.

The top-root ratio was rather small in plants deficient in nitrogen, which is in agreement with the findings of various workers. In this regard it could be stated that the top-root ratio can be changed more or less markedly depending upon the severity of the deficiency. In one case with *Zea,* the plants deficient in nitrogen had a higher top-root ratio than those deficient in phosphorus (table III), while in another case the reverse was true. In these cases the stomata were more responsive in the plants with a larger top-root ratio.

In the cultures deficient in nitrogen or phosphorus, the veins are closer than in plants grown in full nutrient solution or one lacking in potassium. This results in considerable difficulty in obtaining large strips from plants deficient in nitrogen or phosphorus.

The deficiency symptoms are clear-cut in all of the plants used except in *Pisum.* Most plants show the effects of deficiency very markedly, and it is easy to pick out a plant lacking a certain nutrient. In general, recovery takes place when the deficient element is added, except in the older leaves or where the deficiency is very severe and far advanced.

B. STOMATAL BEHAVIOR

In the preliminary experiments, the soil was not mixed with river sand (to make it a poor sandy loam soil), and, as a result, no definite deficiency symptoms were observed. Here the plants were of a healthy green color in all the treatments, but those with full nutrient and those deficient in potassium were often slightly taller. The stomatal behavior in these plants did not show any definite differences. The differences obtained were so small as to be within the range of experimental error. Figure 4 shows the result of one of the experiments of this class with *Zea mays.*

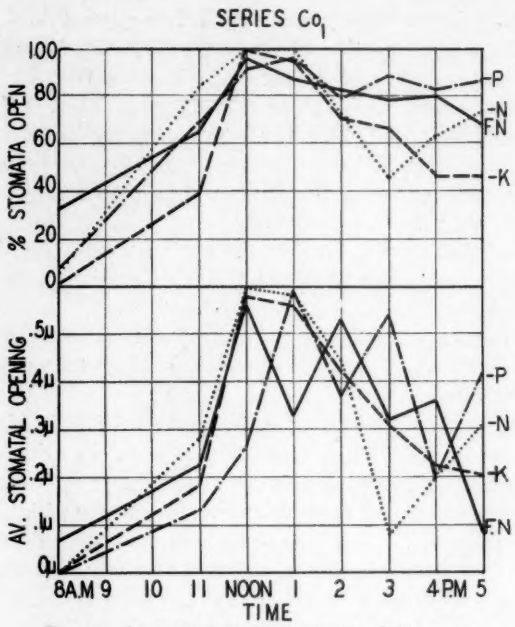

FIG. 4. Stomatal behavior in leaves of *Zea mays*.

The conclusion that stomatal behavior is not markedly affected by nutrient deficiency unless the plants show some definite signs of deficiency is essentially correct, as shown by an experiment with *Pisum* (fig. 5). In this experiment, the roots grew out of the flower pots and obtained nutrients from the cinders with which the greenhouse bench was covered at that time. The cinders had enough potassium for normal growth. The stomatal behavior was practically normal in this case.

The cultures with no nitrogen added (fig. 5), even though well sterilized at the start, became infected by legume bacteria so that abundant root nodules were formed. (The infection, as shown by the size and number of nodules, was greater in plants not supplied with nitrogen as nutrient than in the plants supplied with nitrogen.) The growth in the plants supplied with nitrogen by bacteria only was more like that of normal plants than is the case with plants really deficient in nitrogen. In this case, the stomatal behavior showed a trend toward that of the healthy plants. Although the nitrogen was supplied to these cultures through the nodule organism, it was not enough to make the plants grow as vigorously as when the full-nutrient solution was applied. The stomatal responses were not so quick as in the plants with the full nutrient or those deficient in potassium. This was especially true in the early daylight hours.

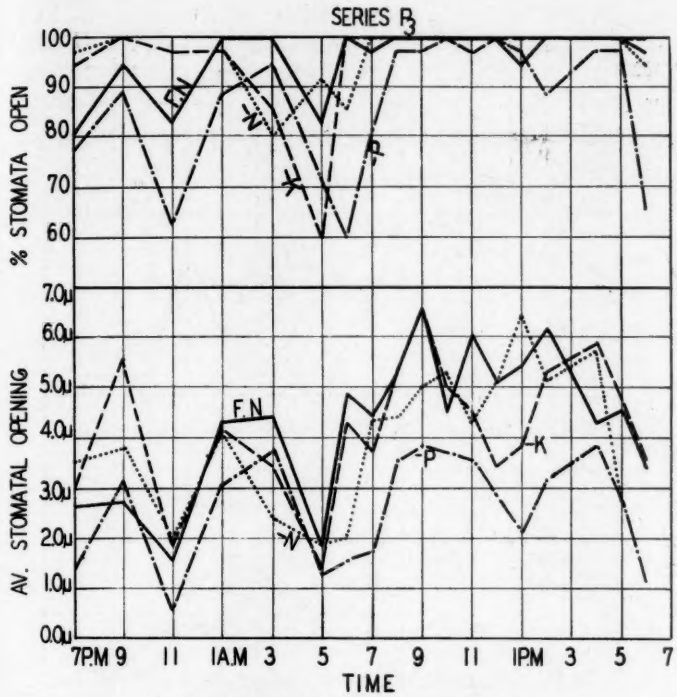

FIG. 5. Stomatal behavior in leaves of *Pisum*.

The cinders were not rich in available phosphorus and the stomatal responses were sluggish, especially in light, in plants where this element was not supplied. In this case, even though the plants showed no specific deficiency symptoms, they were decidedly affected in their growth.

In other sets of experiments no cinders were left on the bench, and most of the series, except *Pisum*, showed clear-cut deficiency symptoms. Whenever these symptoms were obtained, stomata were found to be decidedly subnormal in their responses to the environmental conditions, especially to light. The data of this type, from two of the experiments with *Phaseolus*, are given in figures 6 and 7.

In the first experiment (fig. 6), when all the nutrients were supplied, the stomata showed quick response to changes in light in the morning. Rapid closure of stomata took place with the first signs of wilting (about noon). As the plants recovered from wilting, opening took place. The plants deficient in potassium showed the same general behavior but it was less marked than in the case of the full-nutrient cultures. The leaves of plants with no

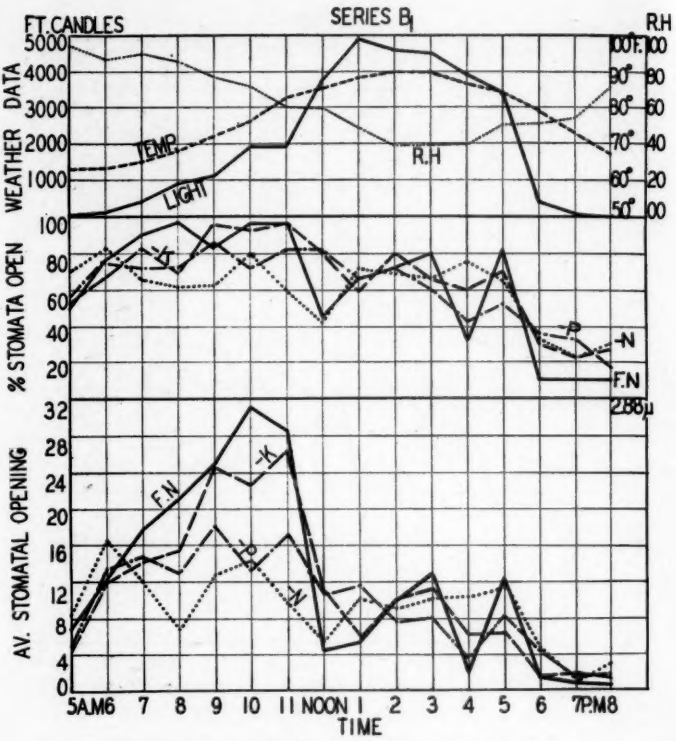

FIG. 6. Stomatal behavior in leaves of *Phaseolus*.

phosphorus or nitrogen were flaccid to the touch at about noon but showed no visible signs of wilting. Here the stomatal responses were slight compared to the other two. In the case of these two cultures, there seems to be no relation between light and stomatal behavior. Some other factor seems to be controlling the stomatal responses.

In the second experiment (fig. 7), the plants were not watered at the beginning as in the previous one. In the plants with full-nutrient supply stomata responded quickly to the changing light conditions. With the onset of wilting, the stomata closed rapidly. At this time (8 A.M.), all the plants were watered, hence they recovered from wilting and the stomata opened.

The plants deficient in potassium showed the same general stomatal behavior but the variations were less marked. The plants deficient in phosphorus or nitrogen showed narrower opening, as usual, though the trend in general response was similar to other cultures.

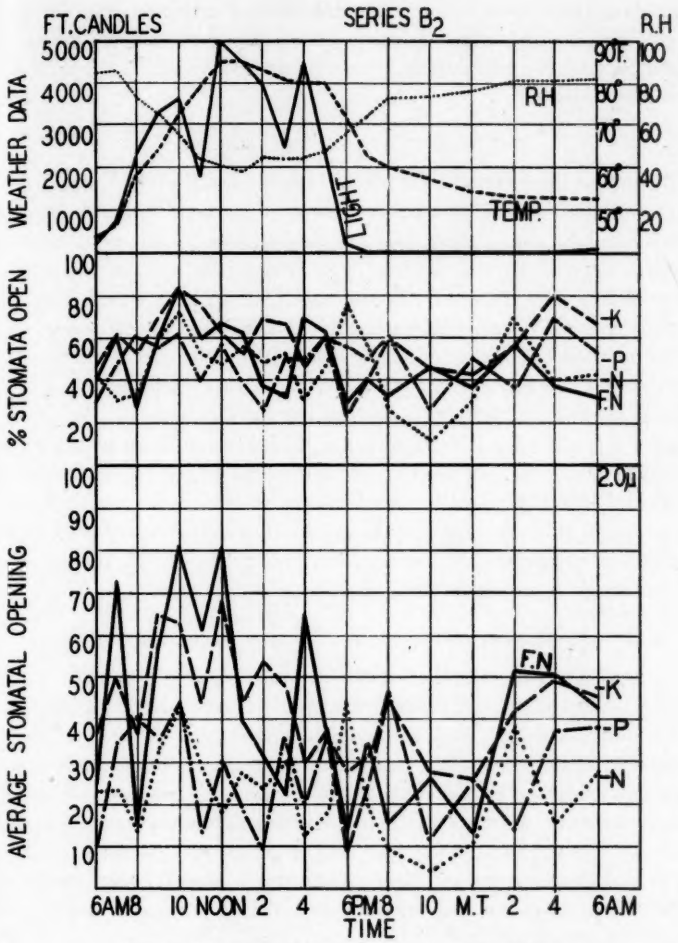

FIG. 7. Stomatal behavior in leaves of *Phaseolus*.

About 11 A.M. (fig. 7), the sky became cloudy. All cultures showed similar stomatal responses. The light was bright again at 12 M. and all cultures except those deficient in nitrogen showed stomatal opening. In this last case the opening response was quite sluggish. At 1 P.M., the plants in full nutrient cultures showed signs of wilting while those deficient in potassium or phosphorus appeared flaccid to the touch but did not show visible signs of wilting. The stomatal activities were what would be expected from previous experience.

The data from these two experiments with *Phaseolus* represent typical data obtained in other experiments with *Tradescantia* and *Pisum* in water cultures, as well as in experiments in soil cultures with *Nicotiana, Zea,* and several other plants.

C. Night opening of stomata

Throughout the course of the present work (figs. 5 and 7), night opening of the stomata has been noted consistently in many plants, notably in *Phaseolus* and *Pisum.* The writer has been unable to find night opening in *Zea,* which agrees with the results of Loftfield (**16**). This phenomenon is not due to any experimental error as some claim, but is real, because the writer has not only seen night opening of stomata that were removed by the strip method but also of stomata observed by the direct visual method. Scarth *et al.* (**32**) have confirmed the truth of this statement.

The *Pisum* plants deficient in potassium have often shown a larger percentage of stomata with wider apertures at night than those plants grown in other cultures (fig. 5). If the CO_2 content of the guard cell vacuome affects the stomatal behavior, then the explanation of Gregory and Richards (**8**) may be true in this case, for they found a higher respiratory rate in plants with deficient potassium. Unfortunately, the technique of these investigators is not above reproach, so nothing definite can be said.

D. Effect of excess potassium on stomatal behavior

In one of the experiments with *Nicotiana* (fig. 8), the pots were half buried in cinders at the beginning. As the pots were a little larger (6-inch) than the standard 4-inch pots, the nutrient supply was doubled. The plants not supplied with potassium obtained an adequate amount of it from the cinders and made the best growth, while the plants supplied with all of the nutrients showed a mottling of their lower leaves, which was followed a little later by shriveling and drying. Hill, Davis and Johnson (**10**) have noted in chrysanthemums a similar injury due to excess of potassium. Apparently, then, excessive and deficient amounts of potassium produce somewhat similar symptoms.

In this case, the total stomatal opening was practically the same in cultures not supplied with potassium as in those cultures receiving the full nutrient supply, but the stomata were more responsive in the former. This may explain the findings of Gassner and Goeze (**7**) who, when they used three different amounts of potassium supply, found that the highest carbon dioxide assimilation occurred with one-twentieth of the normal supply. With increasing concentration, they obtained a decrease in assimilation. They also found a decrease in assimilation with a deficiency of potassium.

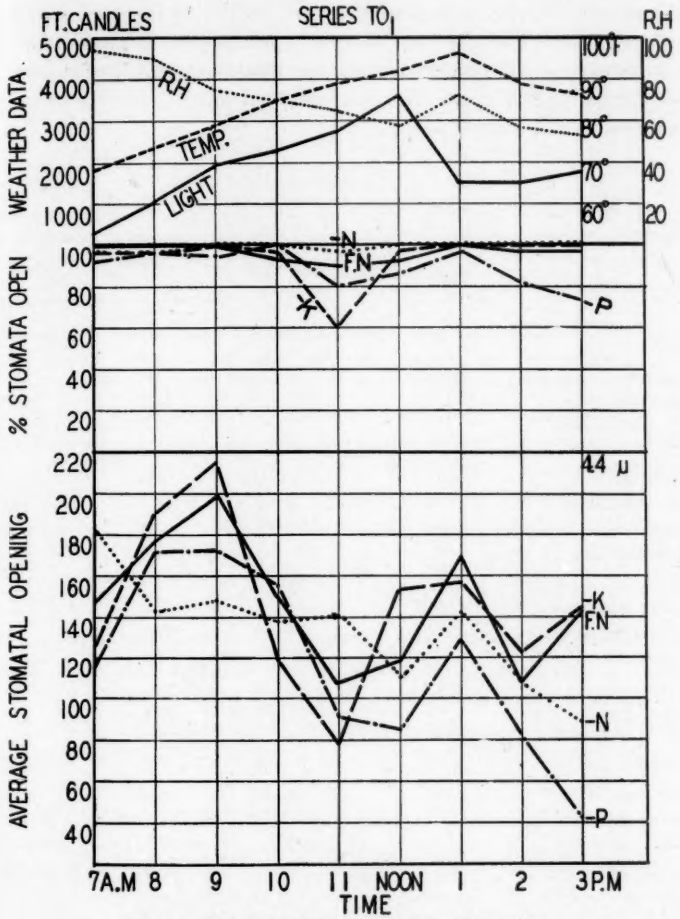

FIG. 8. Stomatal behavior in leaves of *Nicotiana*.

In this series, cultures deficient in phosphorus or nitrogen showed less wide stomatal opening than was found in the cultures discussed above. The total opening was low in cultures deficient in phosphorus as compared with those deficient in nitrogen. This again showed a correlation between the size of the plants and their stomatal behavior.

E. EFFECT OF NUTRIENT DEFICIENCY ON STOMATAL SIZE

Casual observations showed no appreciable differences in the sizes of stomata from variously treated plants. In one of the experiments with

Nicotiana, very careful measurements were made. In this case not only the size of the stomata was noted but also the width of the aperture. The results were surprisingly different from what one would expect. The stomata were slightly larger in the plants supplied with deficient nutrient solutions than in the normal ones (tables I, II).

TABLE I

SIZE OF STOMATA IN *NICOTIANA* (SERIES TO2)

TREATMENT	SIZE OF APERTURE*	NO. OF STOMATA MEASURED†	SIZE* OF STOMATA IN MICRONS	
			AV. WIDTH	AV. LENGTH
	μ		μ	μ
Full nutrient	0–0.2	4	30.0	30.5
Potassium-deficient	"	12	34.3	31.7
Phosphorus-deficient	"	29	35.7	32.0
Nitrogen-deficient	"	1	34.0	26.0
Full nutrient	1–1.2	4	34.1	31.7
Potassium-deficient	"	4	31.7	34.8
Phosphorus-deficient	"	26	37.3	33.5
Nitrogen-deficient	"	10	38.6	35.0
Full nutrient	2–2.2	53	34.8	33.2
Potassium-deficient	"	41	34.6	34.2
Phosphorus-deficient	"	44	37.5	34.2
Nitrogen-deficient	"	50	36.4	35.9
Full nutrient	3–3.2	21	35.5	33.9
Potassium-deficient	"	23	35.4	34.8
Phosphorus-deficient	"	9	38.4	35.3
Nitrogen-deficient	"	14	37.1	35.7
Full nutrient	4–4.2	42	36.0	34.8
Potassium-deficient	"	38	36.1	35.2
Phosphorus-deficient	"	25	37.4	35.7
Nitrogen-deficient	"	25	37.8	36.6

* Measurements of stomatal aperture and stomatal size in tables I and II were obtained with a filar micrometer (Bausch and Lomb 10× ocular) and a 1.8-mm. oil immersion objective.

† These data were obtained from strips obtained in series TO2 during a period of 13 hours (7: 00 A.M. to 7: 00 P.M. inclusive).

These data apparently contradict the findings of LUTMAN (**19**), who observed that with nutrient deficiency the stomatal size decreased in many cases. It seems that the technique used in obtaining the data may contribute to this contradiction. It has been emphasized before that, although the plants showed deficiency symptoms, they did not die but often flowered (fig. 10),

TABLE II

AVERAGE STOMATAL SIZE* IN *NICOTIANA* (SERIES TO2)

TREATMENT	NO. OF STOMATA MEASURED	SIZE OF STOMATA	
		AV. LENGTH	AV. WIDTH
		μ	μ
Full nutrient	124	34.1	32.8
Potassium-deficient	118	35.6	34.1
Phosphorus-deficient	133	37.3	34.2
Nitrogen-deficient	100	40.8	33.8

* Stomatal measurements for each treatment in table I were averaged without regard to the size of the stomatal aperture and are reported in this table (II).

and fruited to a certain extent, *e.g.*, in *Pisum* and *Phaseolus* (table VI). Also, the stomatal measurements were obtained from the third or fourth leaf from the tip of the stem. LUTMAN's data were obtained from comparatively immature plants so that his choice of leaves was limited, with the result that he could not use a definite leaf or portion of a leaf, as was done in the writer's experiments. Owing to this and other reasons, the data are not comparable and the whole problem remains unsettled.[1]

F. EFFECT OF NUTRIENT DEFICIENCY ON STOMATAL DISTRIBUTION

It has been the experience of the writer that the plants treated with all of the nutrients showed a much more uniform distribution of their stomata than the deficient plants. This was especially true of the plants deficient in nitrogen, in which the stomata showed a tendency towards arrangement in groups instead of being evenly distributed. In other deficient cultures this was true, but to a lesser degree.

In figure 9 are given data on the distribution of stomata in one of the interesting cases discussed in section D. Here the full nutrient plants had an excess of potassium, while the plants supposedly deficient in this element got enough of it from the cinders. Stomatal behavior showed the effect of excess potassium (fig. 8). It will be seen that the plants receiving adequate potassium show uniform distribution of their stomata, while the plants with excess potassium do not show as uniform stomatal distribution. But the cultures deficient in phosphorus or nitrogen show a very uneven distribution of stomata as compared with the first two.

In these experiments it has been found that the average number of stomata per unit area of the leaf is not very much affected. The variations are not

[1] The problem of size of stomata and other cells, in these variously treated plants, is being studied by Dr. ERNST ABBE and hence the writer has not developed the subject further.

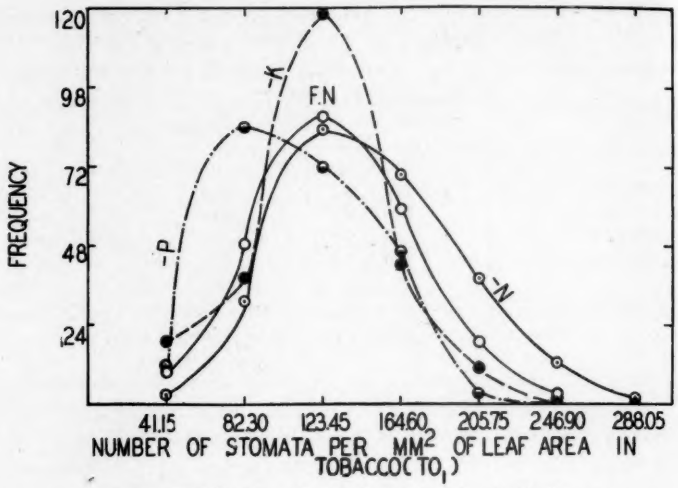

FIG. 9. Distribution of stomata in leaves of *Nicotiana*.

large and show no definite tendency towards decrease or increase with a deficiency of nutrients. The observations of PLEASANTS (**27**) are verified in this regard. The data presented in table III are from the experiment discussed in the above paragraph.

TABLE III

NUMBER OF STOMATA IN *NICOTIANA* (SERIES TO1)

TREATMENT	AV. NO. OF STOMATA PER MM.² OF LEAF AREA
Full nutrient (actually had excess of potassium)	130.5
Potassium-deficient (obtained potassium from cinders)	121.0
Phosphorus-deficient	114.8
Nitrogen-deficient	149.0

G. CORRELATION BETWEEN STOMATAL BEHAVIOR AND OTHER PERFORMANCES OF THE PLANT

In the plants studied it was noted repeatedly that there was a definite relationship between the size of the plant, the yield of the fruit, the water requirement, and the stomatal behavior (tables IV, V, VI, and fig. 10). The plants grown with all of the nutrients were generally larger, had heavier yields (table VI), and a lower water requirement (table IV). The stomata also showed active responses to changes in environmental conditions (figs. 6, 7).

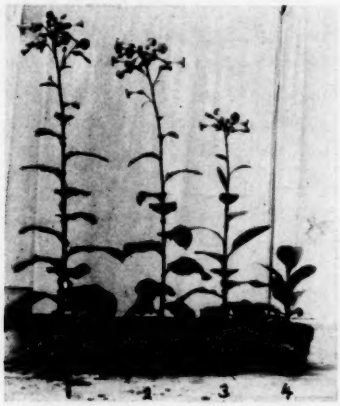

FIG. 10. Size and appearance of *Nicotiana* plants (series TO2) when grown in culture media lacking various nutrients.

1. Plants supplied with full-nutrient solution
2. Plants deficient in potassium
3. Plants deficient in phosphorus
4. Plants deficient in nitrogen

TABLE IV

WATER REQUIREMENT IN *ZEA* (SERIES C2)

TREATMENT	FRESH WEIGHT		TOP-ROOT RATIO	AMOUNT OF WATER LOST IN THREE WEEKS	WATER REQUIREMENT PER UNIT FRESH WT.	
	TOP	ROOT			TOP	ROOT
	gm.	*gm.*		*gm.*		
Full nutrient	81.60	24.38	3.34	377.33	4.624	15.47
Potassium-deficient	68.33	36.13	1.88	413.00	6.044	11.43
Phosphorus-deficient	11.82	9.60	1.22	145.66	12.323	15.17
Nitrogen-deficient	7.27	6.43	1.13	181.00	24.896	28.15

When the plants are a little smaller, as in the cultures deficient in potassium, the yield is less, and there is an increase in water requirement. The stomatal response is not so great as in plants supplied with all of the nutrients (table V).

With plants deficient in phosphorus or nitrogen, the order of stomatal aperture and response, water requirement, and yield varies, depending upon the size of the plants. If the plants deficient in phosphorus are smaller, then their water requirement is larger, their yield is less, and their stomatal response is sluggish. If they are larger than the plants deficient in nitrogen, these data are reversed.

TABLE V

AVERAGE STOMATAL OPENING IN *PHASEOLUS* (SERIES B1)

TREATMENT	AVERAGE STOMATAL OPENING PER HOUR IN MICRONS*
	μ/hr.
Full nutrient	1.08
Potassium-deficient	0.99
Phosphorus-deficient	0.86
Nitrogen-deficient	0.82

* Measurements were made with a filar micrometer; observations were from 5 A.M. to 8 P.M. inclusive.

TABLE VI

YIELD IN *PHASEOLUS* (SERIES B3)

TREATMENT	TOTAL YIELD	NO. OF PLANTS USED	AV. YIELD PER PLANT
	gm.		*gm.*
Full nutrient	584.1	24	24.337
Potassium-deficient	489.6	25	19.584
Phosphorus-deficient	123.3	25	4.932
Nitrogen-deficient	110.0	25	4.400

It seems, then, that there is a clear relationship between stomatal behavior and the general metabolism of the plant. The stomatal behavior is conditioned by the vigor of the entire plant, and is not independent of it. The same conclusion is arrived at when one considers the effect of water relations of a plant on its stomatal behavior.

Discussion

From the data obtained during the course of the present investigation, it seems fairly clear that whenever the plants are affected by nutrient deficiency so that definite deficiency symptoms appear the stomatal behavior is changed. This change, in general, tends towards a less active response by the stomata to light, water, and other environmental factors. This low response is produced not only by a deficiency of these nutrients but also by an excess of potassium. This is especially true when this excess supply of nutrient causes injury of some sort. Such low response appears to be connected with the general metabolism of the whole plant. (It is reasonable to conclude from this that any factor that may affect the general metabolism of a plant may affect the stomatal behavior.)

From the work of SCARTH (31) and others it is fairly apparent that the pH of the guard-cell vacuome in some way conditions stomatal behavior.

Explanations for the possible effect of nutrient *deficiency* on stomatal behavior will revolve around this fact.

At first glance, it seems as if nutrient deficiency may change stomatal mechanism itself, *i.e.*, change the kind and amount of proteins in the guard cells. This would be true in the case of plants grown in culture media in which the plant growth is almost completely restricted, as in the experiments of LUTMAN (**19**). But such a viewpoint does not seem probable for the experiments reported here, as the stomata of plants from all the cultures have a capacity to show active response, as seen from the wide opening when placed in dilute ammonium hydroxide solution. Moreover, the stomata are sometimes a little larger in these deficient plants (*cf.* section E). Furthermore, the responses do not seem to be related very closely to any visible changes in amount of carbohydrates or of protoplasm.

Another explanation suggested by PLEASANTS (**27**) and supported by the work of SCARTH (**31**) and his collaborators (**32**) is interesting and more probable. This explanation is based chiefly on the fact that there exists a correlation between stomatal responses and fluctuations in the pH of the guard-cell vacuome. If the photosynthesis is higher in full-nutrient plants, the shifts in pH should be greater and, as a result, stomatal responses should be more pronounced. If the nutrient deficiency in some way reduced this photosynthetic activity, the shifts in pH should be small, giving lethargic responses by the stomata.

Unfortunately, studies of the effect of nutrient deficiency on plant processes like photosynthesis and respiration show no general agreement. To LUNDEGÅRDH (**18**) this difference in results seems to be due to the use of different plants and of leaves of different ages. The writer's own experience has shown that use of leaves of different location and different age can partly account for these contradictions. Yet, the effect of nitrogen deficiency on photosynthesis seems to be proved to a reasonable degree. It has been shown that nitrogen deficiency decreases the amount of chlorophyll. According to FLEISCHER (**6**), there is a decrease in the rate of photosynthesis with the decrease in chlorophyll content in the alga *Chlorella*.

The lower water requirement of the plants grown in well-fertilized soils is due to the fact that during the period when water loss is rapid, photosynthesis is very high. A slight decrease in the water content of the leaf most probably will not hinder greatly the normal rate of photosynthesis. This has been shown to be true in the case of *Pyrus malus* by CHILDERS (unpublished data). The water loss from stomata becomes less due to a reduced diffusion gradient. When wilting takes place, stomata close rapidly and thereby almost completely stop stomatal transpiration. But the translocation of the accumulated photosynthate will continue. The leaf is then ready to start photosynthesis at a very high rate when the leaf recovers from wilting and the stomata open.

In the case of cultures deficient in nutrients, on the other hand, there are smaller leaf surfaces, fewer leaves per plant, and a relatively larger root system as compared to the amount of top growth. Here the diffusion gradient remains steep during the course of the day resulting in excessive water loss. The amount of photosynthate manufactured during this time will be comparatively less for the amount of water transpired, *i.e.*, the amount of water transpired per unit of dry weight produced (or the so-called water requirement) will be large.

Summary

1. In the present investigation the possible effects of nutrient deficiency on stomatal behavior were studied in *Zea, Pisum, Phaseolus, Nicotiana, Tradescantia,* and other plants. The nutrients studied were potassium, phosphorus, and nitrogen.

2. Stomata observed were those in the distal third of the lower surface of the third or fourth leaf from the growing stem apex.

3. All the methods available for the study of stomata were carefully tested. The direct visual method and LLOYD's strip method were found to be the best. Of these two, the strip method has many advantages over the direct visual method. Certain sources of error in LLOYD's original strip method have been done away with and other improvements in this method are suggested.

4. On the whole, the general deficiency symptoms are similar to those described by other workers.

5. Whenever the plants showed striking deficiency symptoms, stomatal behavior was decidedly subnormal. In the full-nutrient cultures, the plants showed their stomata to be more responsive to changing environmental conditions than when deficient in either potassium, phosphorus, or nitrogen.

6. Night opening of stomata has been observed in practically all the plants used except *Zea.*

7. With excess potassium, the plants were slightly smaller, their lower leaves mottled, and their stomata showed slight lethargic movement.

8. The number of stomata per unit area does not vary greatly, but their distribution is much more irregular in deficient plants. In one case, a tendency towards increased stomatal size in deficient cultures was noted, but in other cases there was no noticeable variation in size.

9. It seems that stomatal behavior reflects the general metabolic condition of the plant. The subnormal stomatal behavior is accompanied by increased water requirement, decreased yield, and decrease in the size of the plants. The average stomatal aperture is proportional to the size of the plant as determined by nutrition, *i.e.*, the widest opening occurs in the largest plant.

10. The viewpoint that leaf activities, *i.e.*, photosynthesis, respiration, and transpiration, control the stomatal behavior appears to be correct. That the condition of stomatal mechanism itself does not change in deficient cultures is indicated by the fact that when placed in ammonium hydroxide solution the stomata in all cultures respond similarly.

The writer wishes to thank Dr. O. F. CURTIS, for suggesting the problem and for advice and direction of the work. Thanks are also due to Dr. LEWIS KNUDSON, and Dr. L. W. SHARP for their assistance and criticism of the manuscript.

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THE CONTINUOUS MEASUREMENT OF PHOTOSYNTHESIS, RESPIRATION, AND TRANSPIRATION OF ALFALFA AND WHEAT GROWING UNDER FIELD CONDITIONS[1]

MOYER D. THOMAS AND GEO. R. HILL

(WITH TEN FIGURES)

Introduction

The apparatus and experimental technique which are described in this paper have been developed to determine *continuously, automatically,* and *throughout the life of a plant,* if desired, the carbon dioxide exchange and the transpiration in a chamber in which plants are growing under field conditions. This discussion gives a general résumé of our methods, including, first, a description of the equipment; second, illustrations of its application to alfalfa and wheat; third, a statistical analysis of the data, showing the magnitude of the errors and variability involved; and fourth, some interesting observations on the relation of light intensity and temperature to the rate of photosynthesis, respiration, and transpiration, which these methods have made clear.

The lack of an adequate method for the rapid and continuous determination of atmospheric carbon dioxide has been responsible for the somewhat limited employment of the carbon dioxide exchange in the measurement of photosynthesis of plants and respiration of both plants and animals. The best methods available—including the gasometric procedure of BENEDICT (**2**); the volumetric method of JOHNSON and WALKER (**5**); the electrometric method of SPOEHR and MCGEE (**8**); and the gravimetric methods—have left much to be desired either of convenience or accuracy for this purpose. Since the atmosphere contains only 310 parts per million by volume of carbon dioxide, the gasometric and gravimetric methods have been handicapped by insufficient material, and the volumetric and electrometric methods have needed more efficient absorbers to permit the use of larger air samples. The latter need recently has been filled by the absorbers of MARTIN and GREEN (**6**), HEINICKE and HOFFMAN (**4**), and THOMAS (**9**). A unique method of determining carbon dioxide exchange has recently been devised by MCALISTER (**7**). After this paper was submitted for publication, a paper by WAUGH (**10**) appeared, describing a hand-operated conductivity appa-

[1] Grateful acknowledgment is made for assistance in carrying out these investigations to the following: Operation of apparatus: LYNN BROWN and J. ALLEN; agronomic observations: L. W. NIELSEN; calculation of results: T. R. COLLIER, D. BONNER, T. BUNKALL, M. CHRISTENSEN, F. EVANS, R. HIRST, S. R. IRVINE, and G. MINER; chemical studies: R. H. HENDRICKS, A. ANDERSON, R. EVANS, H. LINFORD, D. PETERSON, G. F. SOMERS, G. TANNER, and D. WILLIAMSON; records: M. R. BERNSTON.

ratus, based on the same principles as have been employed in the automatic apparatus used in this work. WAUGH refers to a recent paper by HOLDHEIDE, HUBER, and STOCKER,[2] who describe a somewhat similar apparatus.

Description of apparatus

We have worked with six-foot square plots of plants growing in the field under as nearly natural conditions as possible. Over these plots is placed a celluloid-covered cabinet, through which measured volumes of air are passed at rates ranging up to several hundred cubic feet per minute. Continuous and simultaneous measurements of the carbon dioxide content of normal air going into the cabinet and coming from it are made.

THE CO_2 AUTOMETER

The carbon dioxide analytical machine or autometer was described in 1933 in *Industrial and Engineering Chemistry* (9). A diagram of the essential features of this apparatus is presented in figure 1, which illustrates clearly the direction of flow of liquid and gas through the system under the control of the cam-operated automatic valves A to T, inclusive. The autometer has two absorbers, employing 0.005 N sodium hydroxide as absorbent. These absorbers are capable of scrubbing out all of the CO_2 from a stream of air passing at about 300 cc. per minute. This is accomplished by using a sintered glass disk to break up the air stream into very fine bubbles, and a surface tension depressant—normal butyl alcohol—in the liquid, to cause foaming and thus to prolong the period of contact between the bubbles and the liquid. The progress of the absorption is followed by measuring the electrical conductance of the solution at constant temperature with a recording Wheatstone bridge, making use of the fact that sodium hydroxide solution has about twice the conductance of the equivalent sodium carbonate solution.

A motor and reduction gear system, not shown in figure 1, operates an air-pump, and also the two camshafts indicated at the top of the figure. The cams open and close the automatic valves in proper sequence so that the aspiration of the absorbers alternates every two minutes, and the absorbing solution is retained for 8 successive aspirations in each absorber before being drained out and replaced with a fresh charge. The electrical conductance of the solution is automatically recorded each time the absorber is quiescent. An air sample of exactly 600 cc., or any other desired volume, is dispensed to each absorber by a special mercury gas meter in each 2-minute period. The meter consists of two flat steel boxes $15 \times 20 \times 2$ cm. deep, connected through a U-tube. The meter contains enough mercury to fill one box com-

[2] HOLDHEIDE, W., HUBER, BR., and STOCKER, O. Eine Feldmethode zur Bestimmung der momentanen Assimilationsgrösse von Landpflanzen. Ber. d. bot. Ges. **54**: 168–188. 1936.

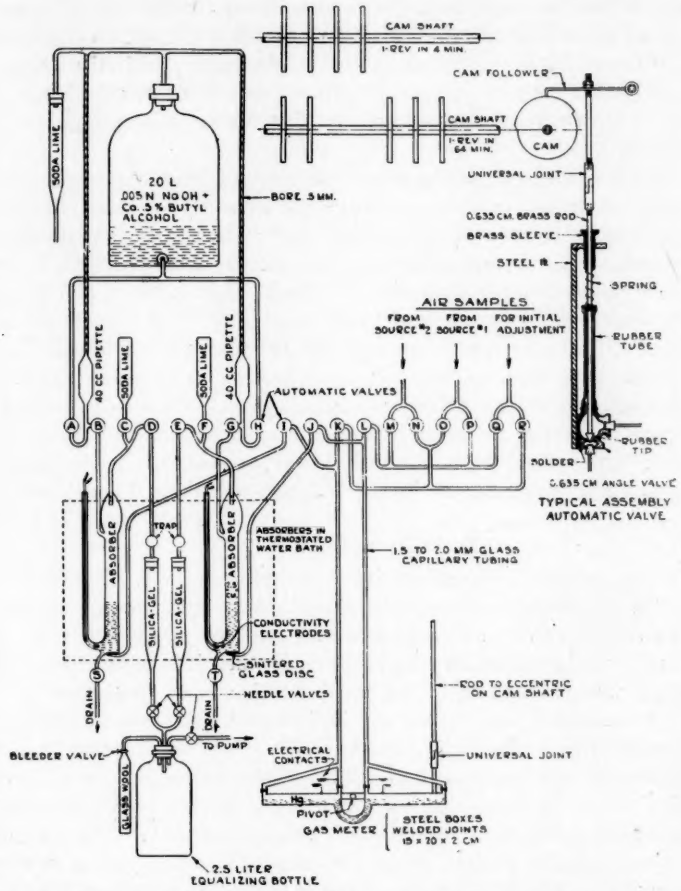

FIG. 1. Diagram of the carbon dioxide autometer. The method of measuring and controlling the flow of liquid and gas is shown.

pletely and leave 600 cc. of free space in the other. The mercury, under the control of valves, D, E, I, J, K, and L, flows alternately from one box to the other, thereby dispensing the air sample before it and drawing in a fresh sample behind it. An eccentric on the camshaft raises and lowers one side of the meter on a pivot so that there is never an appreciable hydrostatic head of mercury in either box. The rate of flow of the gas is adjusted by the suction system so that the entire gas sample is withdrawn from the meter in a few seconds less than the 2 minutes allotted for the operation. The

mercury is then drawn up into the glass capillary tubing above the meter a distance of about 5 cm., depending on the reduction of pressure in the equalizing bottle, which is controlled by the bleeder valve, until the end of the 2-minute period. The electrical contacts on the gas meter permit the registration on the recorder chart of the fact that the complete sample has been withdrawn.

A cycle consists of 16 2-minute periods, giving 8 samples to each absorber, which retains the same absorbing solution throughout the cycle. In the next cycle the absorbers are recharged with liquid and the source of the samples is reversed. This reversal is accomplished by the valves M, N, O, P, two of which are open while the other two are closed. The valves Q and R supply the first sample of air from a large reservoir for the initial adjustment of the solution. Thus by averaging two consecutive cycles any slight machine differences between the absorbers or electrodes can be canceled out and an accurate measure of the difference between the CO_2 concentrations at the intake and outlet of the plant chamber over a 64-minute period can be obtained. This principle of alternating the source of the air samples is also employed when comparing two plant chambers using two autometers.

THE CO_2 RECORDER CHART

A section of the recorder chart is illustrated in figure 2. This chart represents the CO_2 concentrations at the intake and the outlet of a plant chamber between the hours of 4:40 P.M. and 8:15 P.M. on August 6, 1936. The significance of the record may be understood when it is pointed out that A represents the conductance of the spent solution in the first absorber just before it is drained out, causing the indicated conductance to fall to zero. B represents the corresponding conductance of the second absorber. The conductance of the two fresh and initially-adjusted solutions are shown at C and D. Then the conductance decreases in steps after each pair of 2-minute absorptions until the cycle is completed at E and F. These solutions are drained out, the sources of the air samples reversed, and a new cycle commences at G and H. The track at the right margin proves that a full air sample was dispensed for each absorption. At the beginning of a number of the cycles in this chart the source of the samples for the individual absorbers—whether intake or outlet of the plant chamber—is designated. Since the volumes of the absorbing solution and the volumes of the air samples are constant, the displacement of the conductance of the solution from C to E and from D to F represent the concentration of the carbon dioxide in the intake and outlet air respectively. For each cycle, the intake and outlet concentrations have been calculated and placed upon the graph; also the percentage change of concentration of the air stream on passing through the plant chamber. Pairs of cycles have been grouped together and averaged

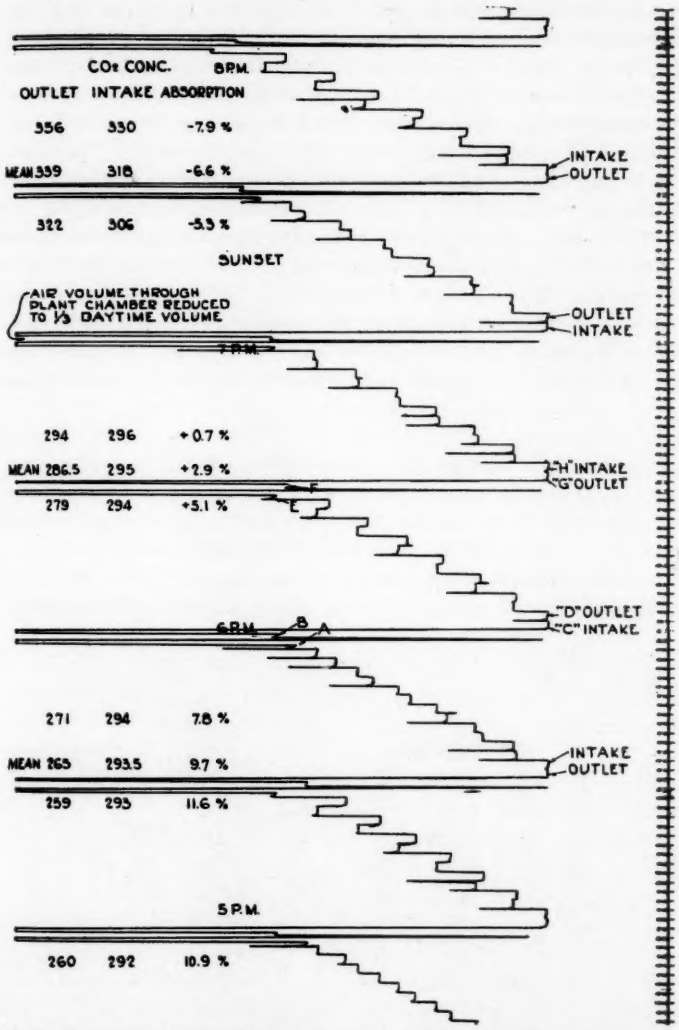

FIG. 2. Sample section of the recorder chart, made during the evening of August 6, 1936. The recorder gives the analyses of intake and outlet air samples from a plot of alfalfa. This chart indicates photosynthesis during sunlight (to 7:05 P.M.). Thereafter it indicates respiration.

to give the values for 64-minute periods. It will be noted that there is a decrease in the positive values of the absorption until 6:30–7:00 P.M., when

the two concentrations were almost equal, indicating a decrease in the amount of photosynthesis with the approach of sunset at 7:20 P.M. In fact, close scrutiny of the record reveals that the compensation point occurred at about 7:05 P.M. Thereafter the amount of carbon dioxide leaving the plant chamber was greater than that which entered, indicating respiration. The air flow through the plant chamber was reduced to one third its daytime value at 7:00 P.M. This accounts for the apparently exaggerated amount of respiration as compared with the earlier assimilation. It should be noted also that the concentration of carbon dioxide in the air rose from about 292 parts per million during the first hour depicted, to 330 parts per million in the last cycle. This rise in the concentration of CO_2 in the air occurs regularly in the evening and the reverse effect is noted regularly in the morning. It is doubtless caused by the depletion of CO_2 in the air close to the ground by photosynthesis in the daytime and its enrichment by respiration at night.

THE PLANT CHAMBER

Figure 3 is a photograph taken September 7, 1936, of a pair of the plant chambers numbered 7-A and 8. The CO_2 exchange of these two plots, which appear to be identical in stand, is presented below. The photograph shows a blower and the 8-inch galvanized pipes leading into the chambers; also tin windows, which permit some access into the interior of the chambers, and the small glass sampling tubes through which samples of intake and outlet

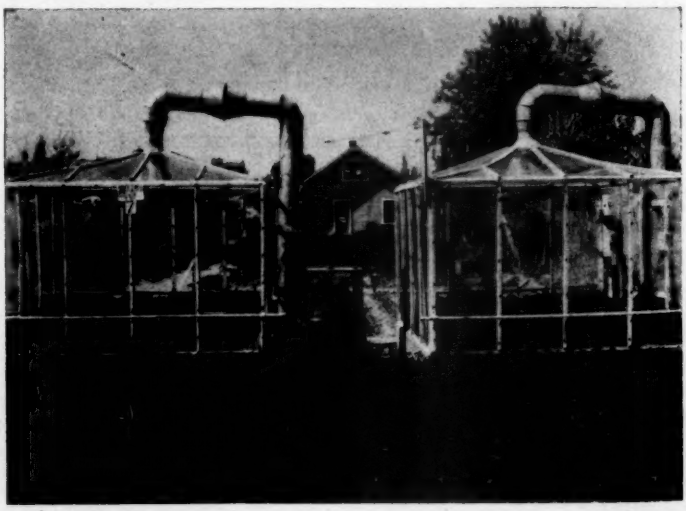

FIG. 3. Plots 7-A and 8 on September 7, 1936, illustrating the plant chamber and blower.

air are taken to the autometers for carbon dioxide analysis. A celluloid window is shown on the intake pipe, through which an anemometer may be read without disturbing the air flow. The exit pipe is just beyond the intake pipe and it also contains an anemometer. These instruments are not removed from the pipes except to interchange them between the different pipes. They are read at frequent intervals and are interchanged daily.

A careful study has been made of the anemometer readings in order to convert the indicated velocity readings into air volumes. For this purpose it has been necessary to determine the channeling of the air in the pipe and also to determine the relations between the average rates of flow on a given cross-section and the volume of air passing through the pipe. These relations have been determined empirically by passing measured volumes of air through the pipes and measuring the velocities with anemometers under conditions simulating those which obtain in the experimental work. In this way it has been found that the pipe containing the anemometer behaves as if it were considerably smaller than its dimensions would indicate. Since the anemometers do not give a reliable indication at low air velocities the practice has been adopted of placing a rubber collar around the instrument, so as to force all of the air to go through it when small air volumes are used. In this way it is possible to measure small air volumes (1000 liters per minute or less) such as are used at night, with the same precision as the larger air volumes (4000 to 7000 liters per minute), which are employed during the day.

By measuring the volume of air entering and leaving the plant chamber it is possible to determine the leakage in the system. This seldom exceeds 10 per cent. of the total volume, and the air volume employed in the calculations is the mean of the intake and outlet volumes.

Respiration and photosynthesis data

Alfalfa

The respiration and apparent photosynthesis values of two plots, nos. 7-A and 8, which were measured continuously and simultaneously for 26 days, from September 4 to October 1, 1936, except for 2 days when the equipment had to be used for other purposes, are illustrated in figure 4, charts A, B, and C. The abscissae represent time. The occurrence of 6 A.M. and 6 P.M. on the different days is indicated. The ordinates represent grams of carbon dioxide absorbed per period of 64 minutes. The values below the zero ordinate indicate respiration; above zero, apparent assimilation.

The symmetry of the curves is striking; also the fact that the independent, although similar, plots show such close concordance. Many pairs of points are coincident, though at times the curves depart from each other slightly.

Seventeen of the 26 days were cloudless and the photosynthesis curves are quite regular and symmetrical. Clouds of different types occur at least

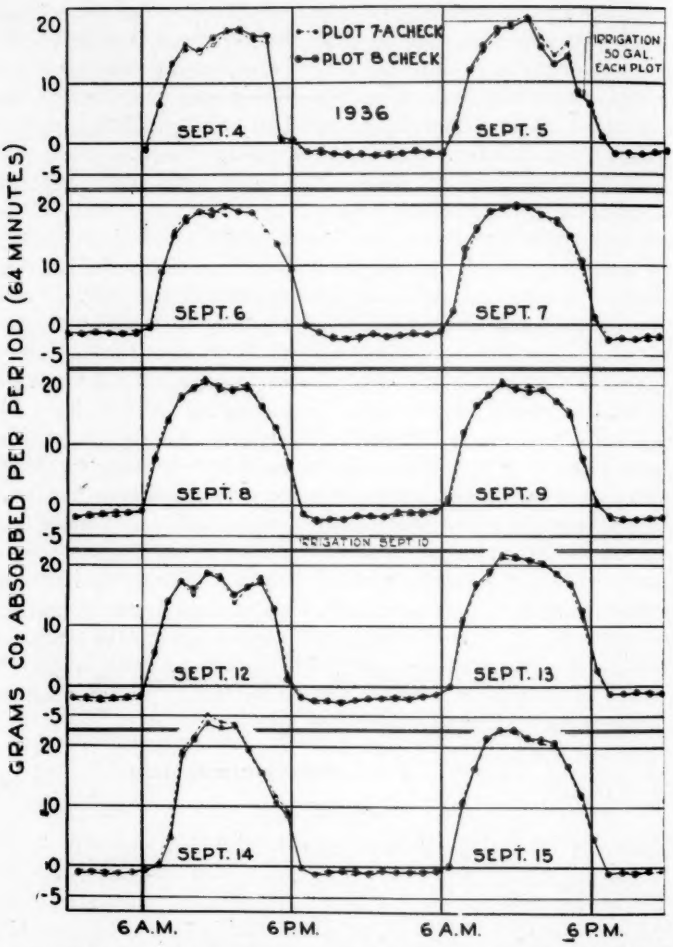

FIG. 4. Chart A. Photosynthesis and respiration curves for alfalfa plots 7-A and 8, from September 4 to September 15, 1936. The values for each 64-minute period of the respiration at night and apparent assimilation during the day on each plot are indicated.

at times on the other 9 days illustrated, and this cloudy weather is reflected by irregularities in the assimilation curves. For example, a very heavy cloud in the late afternoon of September 4 caused photosynthesis to drop almost to zero at 4:30 to 5:30 P.M., and dense clouds in the morning of September 14 delayed the beginning of photosynthesis over an hour. The irregularities

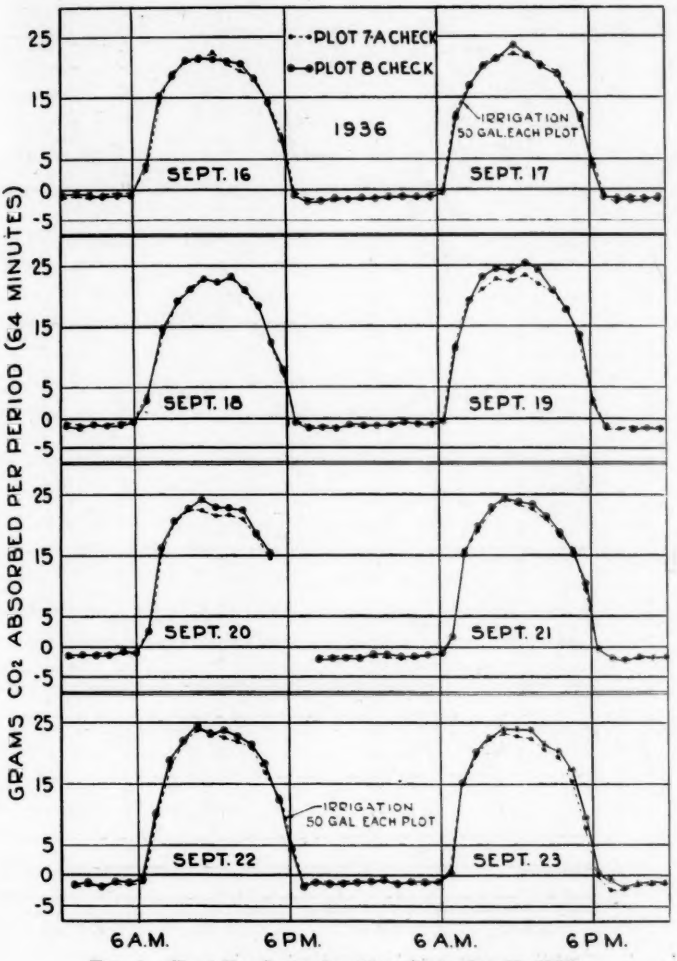

FIG. 4. Chart B. September 16 to September 23, 1936.

on September 5, 12, 25, 26, and 27 were due to intermittent clouds during these days. September 25 and 26 will be referred to again later.

There is a 5-day period between September 19 and 24 in which plot 7-A appears to be less active than plot 8 during part of the day. This condition we believe to be due to failure of irrigation water to penetrate plot 7-A uniformly, with the result that one side of the area was appreciably drier than

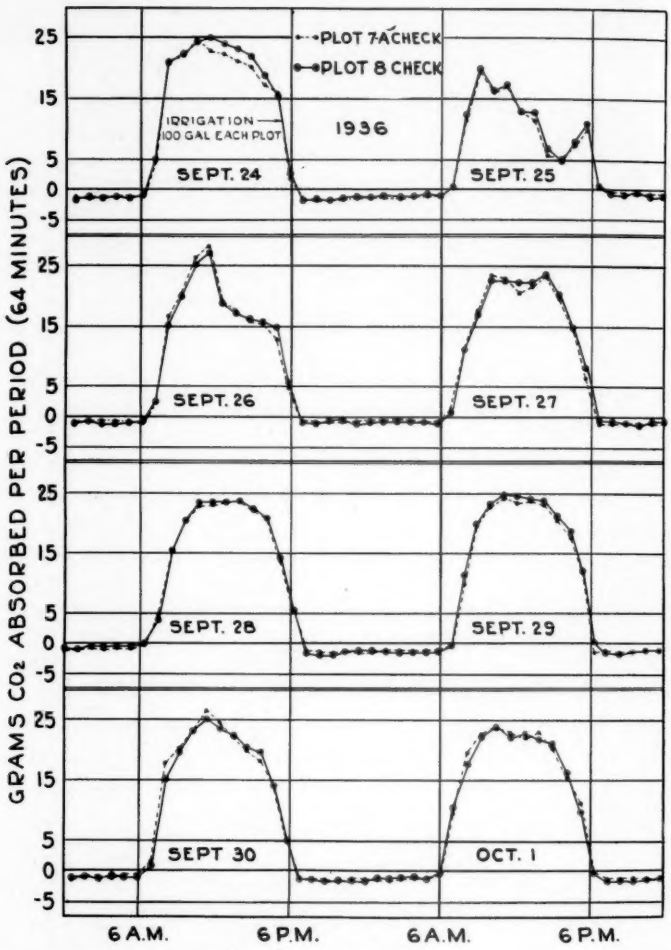

FIG. 4. Chart C. September 24 to October 1, 1936.

the rest. When this plot was heavily irrigated on September 24 the photosynthesis of the two plots quickly came to close agreement again.

The respiration curves are nearly coincident. It will be noted that there is a tendency for the amount of respiration to decrease as the night advances, owing to a lowering of the temperature. In this connection it should be pointed out that no account has been taken in this work of "soil respiration."

A number of observations have indicated that this is a second-order factor, but more work needs to be done on this point.

The data of plots 7-A and 8 are summarized in table I, which is a balance sheet of growth and carbon dioxide exchange. The table gives the total daily apparent assimilation, respiration, and net assimilation, and their ratios, for each day during the period of observation. The total net assimilation is also given. The net assimilation values have been converted into equivalent dry matter, assuming on the basis of chemical analysis that the dry plant substance contained 44 per cent. of carbon. An estimate was made of the amount of dry material on the plot on September 4, when the observations began, by harvesting adjacent areas of alfalfa which appeared to be equivalent in height and density of stand. The plots were harvested on October 2, and the dry matter was determined. In this way it is shown that only 16.4 per cent. to 16.7 per cent. of the total net assimilation could be accounted for as top growth. The remainder probably entered the roots and there was stored. Similar experiments carried out in the summer have accounted for much larger percentages of the carbon dioxide assimilated as top growth. Evidently the low temperatures obtaining in September were conducive to storage of the products of photosynthesis rather than to top growth.

It will be noted that the rate of net assimilation was 15 per cent. greater on September 29 than on September 6, which were comparable days. In this period the estimated increases in total functioning dry matter and leaves were 23 per cent. and 16 per cent. respectively.

Wheat

A few experiments have been carried out with wheat. The variety of wheat used was Utah Experiment Station no. 11544, a very uniform pure-line selection from Hard Federation × Dicklow—a cross made some years previously. The curves of photosynthesis and respiration are quite similar to those obtained with alfalfa. Figure 5 presents the total daily apparent assimilation of a plot of wheat which was under observation from June 6, when the plants were just entering the boot stage, until July 12, when the grain was in the dough stage. This plot consisted of 4 rows of plants with 20 plants in each row. Some indication of the light intensity on the different days is given by the use of letters, which indicate whether the day was cloudless or whether the clouds were light, medium, or dense. On July 8 one row of the plot was harvested to obtain material for analysis and also to establish the amount of plant substance on the plot. Accordingly, on the last 5 days the amount of apparent assimilation has been calculated to a quantity of plant substance comparable with the rest of the experiment, as indicated by the circled points. In spite of the daily fluctuations it is apparent from the chart that the assimilation level of the wheat increases to maximum value at the flowering stage, then decreases through the milk and dough stages.

TABLE I

BALANCE SHEET OF GROWTH AND CARBON DIOXIDE EXCHANGE PLOTS 7-A AND 8 (ALFALFA)

DATE (1936)	TOTAL APPARENT ASSIMILATION CO_2			NIGHT RESPIRATION CO_2			NET ASSIMILATION CO_2		
	PLOT 7-A	PLOT 8	RATIO $\frac{7\text{-A}}{8}$	PLOT 7-A	PLOT 8	RATIO $\frac{7\text{-A}}{8}$	PLOT 7-A	PLOT 8	RATIO $\frac{7\text{-A}}{8}$
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
Sept. 4M†	140.4	144.5	97	18.7	19.2	97	121.7	125.3	97
" 5M	153.5	148.5	103	15.4	15.5	99	138.1	133.0	104
" 6	169.1	174.7	97	16.4	16.7	99	152.7	158.0	97
" 7	166.9	170.5	98	21.2	19.5	109	145.7	151.0	97
" 8	175.6	175.4	100	22.4	20.3	110	153.2	155.1	99
" 9L	166.6	166.2	100	22.8	21.2	108	143.8	145.0	99
" 10M	160.0*	160.0*		22.0*	22.0*		138.0*	138.0*	
" 11	172.0*	172.0*		22.0*	22.0*		150.0*	150.0*	
" 12M	150.7	151.5	99	21.3	22.2	96	129.4	129.3	100
" 13	178.6	181.9	98	11.4	12.1	95	167.2	169.8	99
" 14L	174.5	168.1	104	9.2	9.1	101	165.3	159.0	104
" 15	189.1	191.0	99	9.8	10.0	98	179.3	181.0	99
" 16	182.8	185.3	98	13.0	15.1	86	169.8	170.2	100
" 17	187.5	186.6	101	13.5	14.2	95	174.0	172.4	101
" 18L	183.2	184.8	99	12.8	13.6	94	170.4	171.2	100
" 19	196.1	205.9	96	16.9	16.8	101	179.2	189.1	95
" 20	190.1	200.0	95	16.4	16.3	101	173.7	183.7	95
" 21	193.0	195.8	99	18.9	17.9	106	174.1	177.9	98
" 22	195.6	200.0	98	14.3	14.1	102	181.3	185.9	98
" 23	188.2	199.3	95	17.4	15.1	115	170.8	184.2	93
" 24	190.1	199.8	95	16.6	15.7	106	173.5	184.1	94
" 25D	121.3	126.2	96	8.9	10.0	88	112.4	116.2	97
" 26M	176.9	176.7	100	8.2	9.2	89	168.7	167.5	101
" 27L	182.5	185.2	99	11.3	9.4	120	171.2	175.8	98
" 28	192.4	193.2	99	14.6	15.0	97	177.8	178.2	100
" 29	195.6	202.6	97	14.4	13.4	108	181.2	189.2	96
" 30	194.6	190.0	103	15.6	15.0	104	179.0	175.0	102
Oct. 1	191.2	189.2	101	13.9	15.0	93	177.3	174.2	102
Total	4958.	5025.	98.7	439.	436.	100.7	4519.	4589.	98.5
				Equivalent dry matter			2800.	2845.	
				Dry matter October 2nd			1690.	1725.	
				Dry matter September 4th (estimated)			1230.	1250.	
				Top growth (grams)			460.	475.	
				Top growth—per cent. of total CO_2 assimilated			16.4	16.7	
				Probable root increment (grams)			2340.	2370.	

* Estimated.
† L = light clouds; M = medium clouds; D = dense clouds.

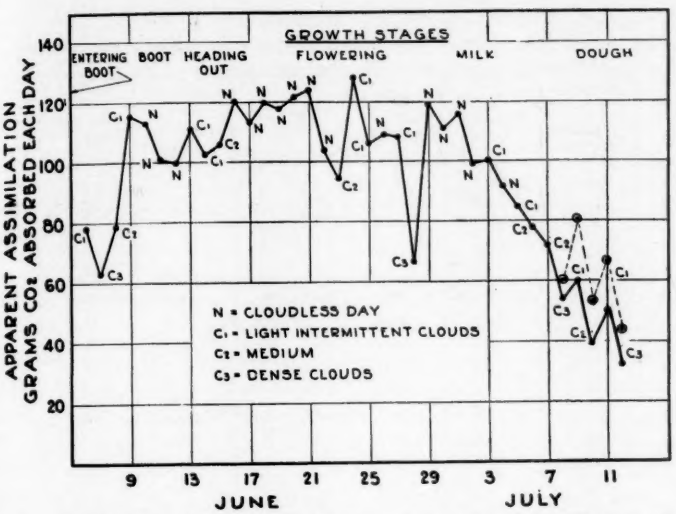

FIG. 5. Total daily carbon dioxide absorption of wheat from June 6 to July 12, 1936. The type of light conditions prevailing on the different days is shown. On July 8 one-fourth of the plot was harvested and the subsequent data have been adjusted to the original quantity of plant substance as indicated by the circled points.

When the experiment was discontinued the assimilation level was only about half of the level during the period of maximum activity. At this time the amount of functioning tissue was greatly curtailed due to the senescence. On July 8 55 per cent. of the leaf blades were dead, including 8 per cent. of the apical, 12 per cent. of the second from the top, and 37 per cent. of the third leaf blades.

Table II is a balance sheet of growth and carbon dioxide exchange on this plot of wheat. The data are presented in the same form as in table I. It will be observed that the apparent assimilation is considerably lower in the wheat plot than in the alfalfa plot. This is due largely to the fact that there was a considerably smaller amount of functioning tissue on the former. On the other hand, the respiration levels of the wheat and alfalfa are approximately the same, which is probably explained by the fact that the night temperatures during June were higher than the night temperatures in September. It is particularly interesting to note that 83.3 per cent. of the total carbon dioxide assimilated is accounted for as top growth, with only 16.7 per cent. which may be assumed to be root increment.

Statistical analysis of the analytical data

The following statistical analysis will consider first the errors that may be expected in the absorption data as a result of errors attributable to the

* Estimated.
† L = light clouds; M = medium clouds; D = dense clouds.

TABLE II

BALANCE SHEET OF GROWTH AND CARBON DIOXIDE EXCHANGE PLOT 6 (WHEAT)

DATE	APPARENT ASSIMILATION	NIGHT RESPIRATION	NET ASSIMILATION	DATE	APPARENT ASSIMILATION	NIGHT RESPIRATION	NET ASSIMILATION
(1936)	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*
June 6	78.6	17.4	61.2	June 24	128.4	20.5	107.9
" 7	62.9	11.6	51.3	" 25	106.8	21.3	85.5
" 8	78.9	7.0	71.9	" 26	109.0	26.0	83.0
" 9	115.6	7.6	108.0	" 27	108.6	14.3	94.3
" 10	113.7	16.6	97.1	" 28	67.6	16.8	50.8
" 11	101.2	21.2	80.0	" 29	119.6	17.5	102.1
" 12	100.5	18.7	81.8	" 30	111.2	21.1	90.1
" 13	111.1	22.8	88.3	July 1	116.8	17.6	99.2
" 14	103.6	25.3	78.3	" 2	99.7	25.6	74.1
" 15	106.3	10.8	95.5	" 3	100.9	22.6	78.3
" 16	120.8	21.8	99.0	" 4	92.3	14.2	78.1
" 17	113.3	25.1	88.2	" 5	85.3	21.7	63.6
" 18	120.0	17.6	102.4	" 6	81.2	20.4	60.8
" 19	118.5	21.3	97.2	" 7	72.8	17.4	55.4
" 20	122.4	13.9	108.5	" 8	60.0*	14.5*	45.5*
" 21	124.4	25.0	99.4	" 9	80.4*	9.7*	70.7*
" 22	104.2	25.2	79.0	" 10	52.9*	19.7*	33.2*
" 23	95.0	23.4	71.6	" 11	66.8*	20.3*	46.5*
				" 12	43.8*	16.9*	26.9*
		Total			3595.	690.	2905.

Total CO_2 assimilated June 6 to July 8	2682.
Equivalent dry matter	1663.
Estimated dry matter less roots on July 8	1744.
Estimated dry matter less roots on June 6	359.
Top growth—June 6 to July 8	1385.
Top growth—per cent. of total CO_2 assimilated	83.3
Probable root increment—per cent. of total CO_2 assimilated	16.7

* Adjusted.

operation of the autometer; and second, deviations in the respiration and assimilation values of two plots measured simultaneously by two autometers. The latter deviations will reflect not only the analytical errors of the two machines, but also the errors of measurement of the air volumes passing through the plant chambers, as well as actual differences in the respiration and photosynthetic levels of the two plots. When the analytical machines were constructed an extensive test was made to determine the differences that might be expected in the carbon dioxide concentrations of the two absorbers when they were sampling from the same source. This extensive

experiment (**9**) indicated an average deviation between the two absorbers of 0.42 per cent. Since that time, some improvements have been made in the apparatus and the mean of over 300 samples of the same sort have indicated an average deviation between the absorbers of 0.25 per cent. This machine error represents about 1 per cent. of the maximum photosynthetic values as our experiments are ordinarily carried out.

The data in figure 4, charts A, B, and C, have been analyzed by recording the difference between the pairs of analyses. The average hourly deviations (without regard to sign) between the two plots, 7-A and 8, ranged on different days from 0.21 gm. to 1.05 gm. during the daytime and from 0.118 gm. to 0.315 gm. during the night. The average daytime value was 0.60 gm., or, if the 5 days when the drought conditions complicated the results are omitted, 0.52 gm., and the average nighttime value 0.18 gm. These deviations represent 1 per cent. to 5 per cent. of the total absorption during the daytime and 5 per cent. to 15 per cent. of the night respiration. If these deviations were entirely due to errors in analysis, their average values would represent machine absorption errors of 0.3 per cent. to 0.5 per cent. in the daytime and 0.7 per cent. at night, *i.e.*, values 1 to 3 times the normal machine error of 0.25 per cent. A similar statistical analysis of another pair of plots amply confirms the values found for plots 7-A and 8.

When the hourly deviations are analyzed by FISHER's method (**3**) of "t" it is found that the differences are significant on September 19, 20, 22, 23, and 24, when drought conditions probably complicated the results. On only two other days, namely, September 14 and September 29, are the differences significant, and on these two days the absolute values of the differences are negligibly small. Significant differences at night occurred on September 8, 9, 11, 21, and 28, but only on September 11 was the average deviation for the night greater than the average value (0.18 gm. CO_2) mentioned above. Evidently these deviations are so small that this equipment should serve to measure extremely small experimental effects.

Influence of light intensity on photosynthesis

The influence of variable light intensity due to clouds was strikingly shown on a number of days in figure 4, charts A, B, and C. Three of these days have been replotted in figure 6 above the curve representing the light intensity as determined with a 10-junction pyrheliometer placed horizontally, and a recording voltmeter. Intermittent measurements of the light intensity with a Weston cell confirmed the values given by the pyrheliometer. The values of the light intensity at normal incidence also have been calculated from the values on a horizontal surface. September 15 was a cloudless day, and the assimilation curves are essentially regular. Their shape is intermediate between the shapes of the light curves at normal incidence and on

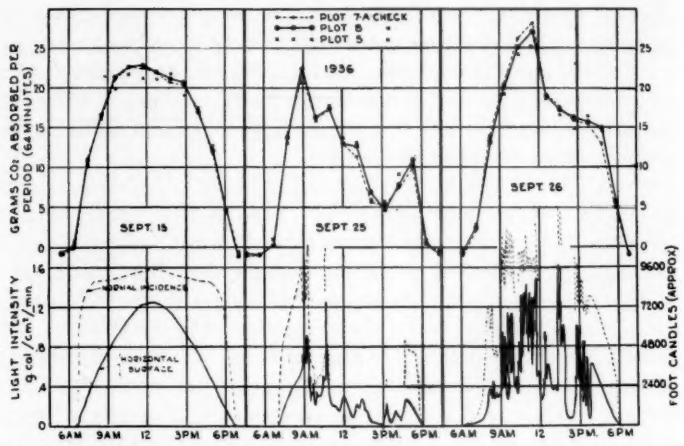

FIG. 6. Relation of photosynthesis to light intensity. Apparent assimilation of 3 different plots of alfalfa on September 15, 25, and 26, 1936, is plotted above the curves of light intensity on the same days. The curves illustrate the rôle of clouds in modifying the photosynthetic values.

the horizontal surface. September 25 and 26 were partly cloudy. It will be seen that the peaks of light intensity correspond closely with the peaks of photosynthesis. Of course, the method of averaging 64 minutes of record in calculating the points on the assimilation curve tends to smooth out irregularities due to too frequent changes in the light. For example, on September 26, a different grouping of the data would have shown a level equivalent to 10 grams between 1:30 and 2:40 P.M., and 20 grams between 2:40 and 3:10 P.M. The flash of sunlight between 1:20 and 1:30 P.M. can be distinguished in the record.

A striking relationship is illustrated on September 26. The photosynthetic maximum is appreciably higher than normal. This appears to be associated with the large fluctuations in light intensity due to frequent obscuring of the sun with clouds. It has frequently been noted that higher than normal apparent assimilation levels are reached when the sun is intermittently obscured by clouds for a few seconds to one or two minutes. This is probably due in part to a lower leaf temperature with attendant lower respiration. It may also be due in part to the orientation of the chloroplasts.

Incidentally it should be pointed out that in addition to the assimilation values of plots 7-A and 8, those for plot 5 also have been placed on the graphs as unconnected crosses. The concordance is excellent, not only in full light, but also in cloud shadow, and particularly so considering the fact that plot 5 is a different variety of alfalfa, and the plants are somewhat older.

Figure 7 illustrates some assimilation curves on cloudless days. The lower curve in the chart to the left represents a plot which is just starting

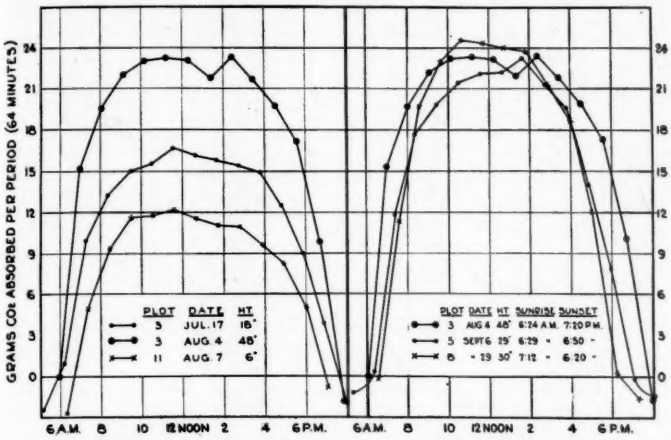

FIG. 7. Relation of stage of growth and length of day to photosynthesis. Apparent assimilation of 3 plots of alfalfa at 3 stages of growth in midsummer (left); and 3 plots of comparable stage of growth at monthly intervals (right).

to grow, the plants being 6–8 inches high. Part of the plants are shaded early in the morning and late in the evening by the base of the cabinet, so that the beginning of assimilation is retarded in the morning and its completion hastened in the afternoon. The upper curve represents a full-grown plot at about the same time, with the plants about four feet tall. The center curve represents the latter plot when the plants were 18 inches high. It is interesting to note that the assimilation level is only twice as great in the plot with plants 48 inches tall and having 3.6 times the weight of leaves, as in the plot with plants 6–8 inches tall, due, at least in part, to a larger proportion of shaded leaves under the fully illuminated surface leaves on the larger plot.

The chart on the right illustrates three more or less comparable plots at approximately monthly intervals. This chart shows the effect of the shorter day, but indicates about the same maximum apparent assimilation level regardless of the fact that the maximum angle of incidence of the sun on September 29 was 47.5° as compared with 66.5° on August 4. Indeed, the apparent assimilation level on the later date was somewhat higher than on earlier dates which was probably a reflection of a lower rate of respiration due to lower temperature.

In figure 8, the effect of clouds in reducing the assimilation level is illustrated. It will be seen that there is no appreciable reduction in the rate of assimilation as long as the light intensity exceeds about 52 per cent. of its

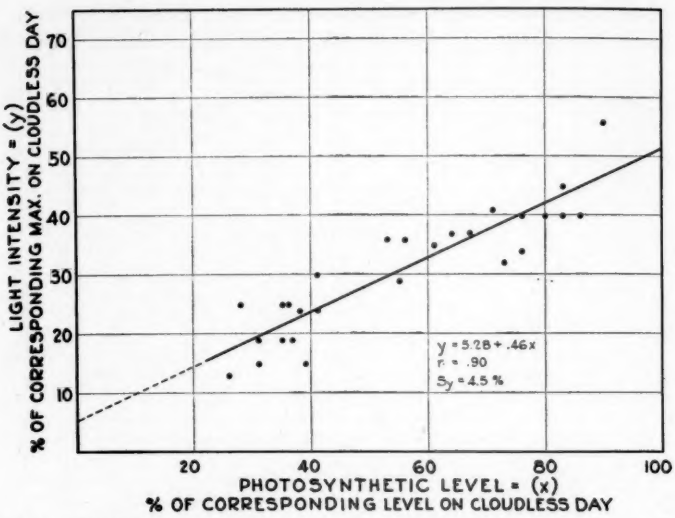

FIG. 8. Relation of photosynthesis to light intensity. The influence of clouds of different densities in reducing the photosynthetic level of alfalfa.

maximum value. The curve seems to indicate a linear relationship between the photosynthetic level and light intensities below 52 per cent. of the normal maximum at Logan, Utah.

Effect of temperature on respiration

Figure 9 illustrates the effect of temperature on the respiration level and is derived by a consideration of the respiration values on a number of different nights in September. During this time there was very little top growth on the plot, and accordingly the quantity of vegetation is substantially constant over the period from which the respiration and temperature values were selected. It will be seen that the respiration level increases about fourfold with a temperature rise from zero degrees to 20° C., which is about the value expected from other work in the literature.

In a number of other experiments, temperature coefficients of approximately this value have also been obtained.

Transpiration

It seemed desirable to attempt to measure transpiration, in addition to the carbon dioxide exchange in the plant chambers. Accordingly, during the summer of 1936, four units of the Leeds & Northrup relative humidity recorders (**1**) were purchased. This recorder employs a pair of carefully-balanced resistance thermometers, mounted in knife-shaped cases. One of

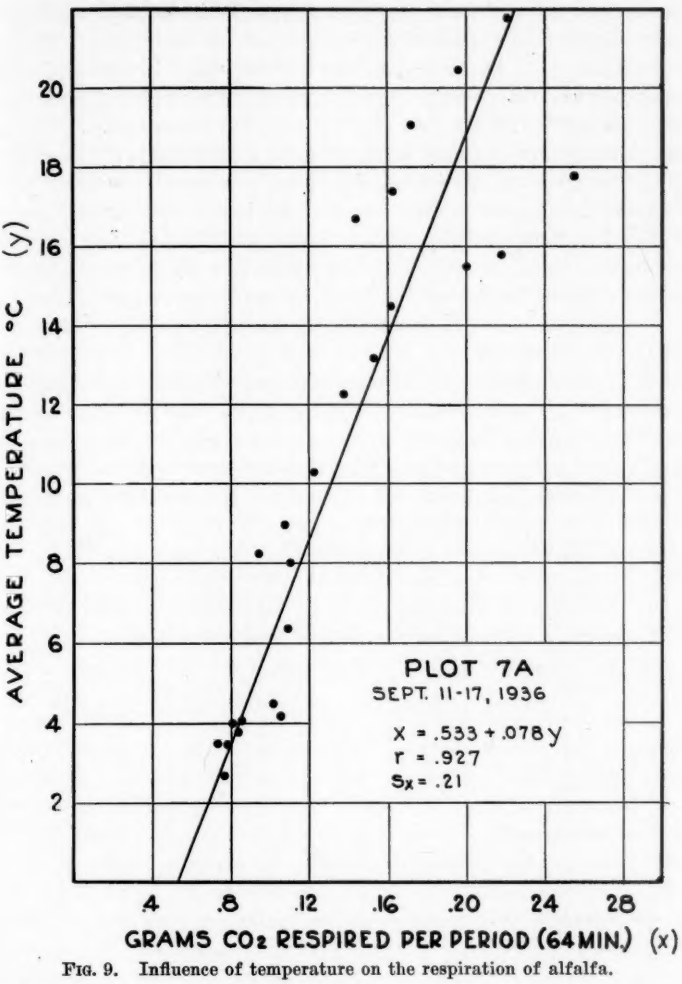

FIG. 9. Influence of temperature on the respiration of alfalfa.

the thermometers is wet by a wick. If wet-bulb temperature is plotted against dry-bulb temperature for a given value of the relative humidity, a straight line is obtained, the slope of which depends on the value of the relative humidity. Relative humidities from 20 per cent. to 100 per cent. thus yield a family of straight lines which on extrapolation intersect in a very limited area near a point corresponding to about – 10° C. The recorder measures the relative resistance of the wet- and dry-bulb resistance ther-

mometers from this point of reference. Thus the instrument reads relative humidity directly regardless of the temperature of the system. The instrument was calibrated by immersing the thermometers in baths of different temperatures and comparing the readings with the hygrometric tables. It was also checked by direct analysis of the air for water vapor. The manufacturer's claim, that the limit of error in the performance of the recorder is within 1.5 per cent. of relative humidity, was confirmed.

Units of the apparatus were set up in the intake and outlet pipes of the plant chamber, dampers being used to insure sufficient air flow over the wet bulb to produce maximum cooling. In addition, a group of resistance thermometers attached to an 8-point Leeds & Northrup temperature recorder made a continuous record of the air temperature at the points where relative humidity was measured.

In calculating the results, the temperature and relative humidity records were averaged by hours and the weight of water vapor which was carried past a given point was estimated on an hourly basis, using the vapor-pressure curve of water and the rate of air flow as determined by the anemometers in the pipes. Figure 10 is presented to illustrate the type of data which have

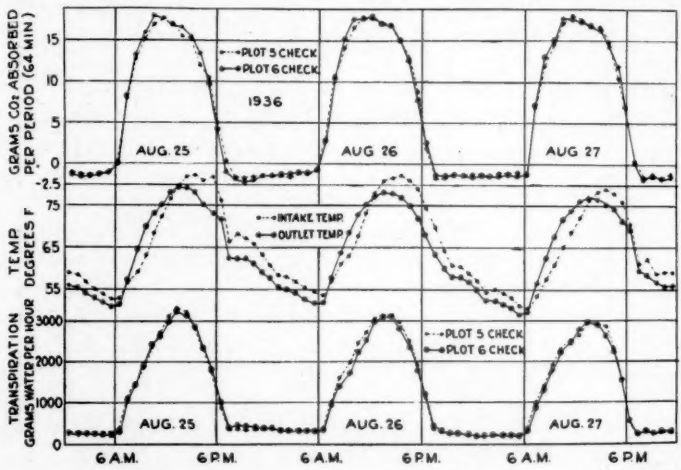

FIG. 10. Curves of respiration and apparent assimilation (upper); average intake and average outlet temperature (middle); and transpiration (lower) of two plots of alfalfa.

been obtained. The transpiration curves of two similar plots, plots 5 and 6, measured simultaneously, are given along with the corresponding carbon dioxide exchange curves and the average intake and outlet temperature curves on 3 cloudless days. The total amounts of water transpired on plots 5

and 6 were 31.1 and 31.3 kilos respectively on August 25; 30.8 and 29.4 kilos on August 26; and 28.2 and 27.6 kilos on August 27. The graphs and data indicate that transpiration can be measured continuously and automatically with an average deviation of less than 5 per cent. It is probable that the equipment is capable of better performance than this. The shapes of the transpiration curves are similar to those of the carbon dioxide curves, except that the former are more pointed and definitely skew, reaching their maximum on a cloudless day at about 2 P.M. There is obviously a close correlation between the rate of transpiration and the temperature, particularly during the day, when the temperature curves are also correspondingly skew. At night the response of transpiration to temperature is less noticeable, though definite. This is due to several causes: first, stomatal closure at night; second, reduction of the air volume passing through the plant chamber to 30 per cent. of the daytime volume; and third, lowering of both the vapor pressure of water and its temperature coefficient when the temperature is lowered. In any case, the graphs show clearly that both transpiration and respiration fall off progressively as the night advances along with the lowering of the temperature.

It should be noted that the outlet temperature exceeds the intake temperature during the morning, but during the afternoon and night the reverse is true. The cooling effect of transpiration is thus manifested at night, and also in the afternoon when evaporation removes more energy from the system than is added by solar radiation. In the morning, when the temperature is lower, the input of radiant energy exceeds the outgo through evaporation.

Summary

1. This paper describes apparatus designed to measure continuously and automatically, the carbon dioxide exchange and the transpiration of plants growing under natural conditions.

2. The carbon dioxide analytical machine or autometer, with its most recent improvements, is briefly described, and a typical section of the recorder chart is presented to show how the apparatus operates.

3. This equipment has been used to measure simultaneously and continuously the carbon dioxide exchange on two similar plots of alfalfa over a period of 26 days. The hourly values of respiration and photosynthesis of the two plots are plotted. Data summarizing the daily apparent assimilation values of a plot of wheat covering a period from the boot stage to the dough stage are also presented.

4. The alfalfa curves show that two similar plots give closely concordant responses to the environment, both as to respiration and photosynthesis. The two curves are analyzed statistically, and it is evident that the apparatus may be used to measure very small effects of an experimental treatment.

5. The influence of clouds on rate of photosynthesis is strikingly shown by the curves. Further, data have been collected which indicate that the rate of photosynthesis of alfalfa is a linear function of the sunlight intensity up to about 52 per cent. of the normal maximum at Logan, Utah. Greater intensities do not increase the rate appreciably.

6. Respiration varies with the temperature, and increases about fourfold in rate between 0° C. and 20° C.

7. The balance sheet of the alfalfa plots shows that only 16.5 per cent. of the net carbon dioxide assimilated could be accounted for as top growth. The remainder was probably stored in the roots. This was a late-season experiment. In the summer a much larger proportion goes into the tops.

8. The wheat data show that the rate of assimilation reaches a maximum in the flowering stage, then falls off in the milk and dough stages owing to the senescence of the plant. The balance sheet accounts for 83.3 per cent. of the net assimilation as top growth.

9. Transpiration has been measured continuously and automatically using Leeds & Northrup relative humidity recorders. The transpiration curves resemble the carbon dioxide exchange curves in shape, but they are more pointed than the latter and also are definitely skew in the daytime. The transpiration curves evidently reflect temperature changes closely; whereas photosynthesis follows more nearly the light intensity. The average deviation of the transpiration values on two similar alfalfa plots was less than 5 per cent.

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RELATION OF SULPHUR DIOXIDE IN THE ATMOSPHERE TO PHOTOSYNTHESIS AND RESPIRATION OF ALFALFA[1]

MOYER D. THOMAS AND GEO. R. HILL

(WITH TWELVE FIGURES)

Introduction

One of the most fundamental processes in the growth of vegetation is that of photosynthesis, by which the principal portion of the plant substance is synthesized. This process involves delicate and complicated chemical reactions under the influence of light—reactions which are readily affected by external conditions. A study of the photosynthetic processes, and also of respiration, in connection with sulphur dioxide fumigations, should throw a great deal of light on the nature of the reactions of the gas with the vegetation.

A carbon dioxide analytical machine or autometer has been made (**6**), which is capable of measuring continuously and automatically the concentration of carbon dioxide at two points in an air stream. The use of this autometer in connection with a celluloid-covered plant chamber (**2, 8**) permits the determination of the carbon dioxide content of a measured volume of air as it enters and as it leaves the plant chamber, thus measuring the rate of photosynthesis during the day of the plants in the cabinet, growing under field conditions, and their respiration at night. By using two units of this equipment, a sulphur dioxide fumigation treatment has been applied to one and the effect of the treatment has been determined by comparing the rate of photosynthesis and respiration of the treated plot with that of a similar check plot.

This paper presents the complete carbon dioxide exchange history of 9 fumigated plots and their corresponding check plots, covering in each case the fumigation period and also periods preceding and following the fumigation. Experimental treatments have been studied ranging from fumigations which produced almost complete foliar destruction, progressively downward to long-continued sublethal dosages of the gas. The experiments are arranged in order of decreasing concentration of applied sulphur

[1] Grateful acknowledgement is made for assistance in carrying out these investigations to the following individuals: Operation of apparatus, LYNN BROWN and J. ALLEN; agronomic observations, L. W. NIELSEN; calculation of results, T. R. COLLIER, D. BONNER, T. BUNKALL, M. CHRISTENSEN, F. EVANS, R. HIRST, S. R. IRVINE, and G. MINER; chemical studies, R. H. HENDRICKS, A. ANDERSON, R. EVANS, H. LINFORD, D. PETERSON, G. F. SOMERS, G. TANNER, and D. WILLIAMSON; records, M. R. BERNTSON.

All of the chlorophyll analyses, and most of the sulphur analyses in this paper were carried out by Dr. R. H. HENDRICKS.

dioxide. The paper also includes the fumigation and yield data of these plots, together with sulphur and chlorophyll analyses of the plants. Other chemical and agronomic data on these plots, and also transpiration studies, will be reported later.

An earlier paper (**2**) compared the effect of leaf destruction by sulphur dioxide fumigation and by clipping on the yield of alfalfa. It was shown that the yield was reduced in proportion to the amount of leaf destruction and that if there was no leaf destruction there was no significant reduction in yield. The two methods of causing leaf destruction—sulphur dioxide fumigation, and clipping off the leaves with shears—produced nearly identical reductions in yield. A second paper (**7**) showed that the quantity of leaf destruction was proportional to the amount of gas absorbed by the leaves, and that an appreciable amount of sulphur dioxide, approximating one quarter of the amount necessary for complete leaf destruction, could be absorbed without any leaf destruction at all. It was also shown that the amount that could be absorbed without injury increased greatly as the rate of absorption decreased, due probably to the rapid conversion of absorbed sulphur dioxide to the thirty-or-more times less toxic sulphate form. The papers cited also described the apparatus and technique for conducting the sulphur dioxide fumigations. Briefly, the procedure consisted in adding the sulphur dioxide from a bottle of the liquid through a capillary flowmeter system to the air stream entering the plant chamber. The air stream was analyzed for sulphur dioxide continuously and automatically (**5**) as it entered and as it left the plant chamber, thus permitting the determination of the quantity of the gas absorbed by the vegetation. In addition, a record was kept of the environmental conditions—light, temperature, and relative humidity. Observations on growth, yield, and leaf destruction were also made.

As a matter of interest, in the charts which will be presented later, the occurrence of clouds has been indicated in a number of cases. It should also be noted that the carbon dioxide exchange data of 4 plots on the same days furnish many examples of quadruplicate records. Attention may be directed to August 23 and 28, 1935, on figures 10 and 12; to August 5, 1936, on figures 3 and 4; and to August 12, 14, and 16, on figures 4 and 5, as striking examples of these quadruplicate records.

Effect of sulphur dioxide on alfalfa as indicated by the carbon dioxide exchange

I. Experiments at Salt Lake City, Utah

A. Effects of fumigations producing nearly complete defoliation.—At the Agricultural Research Laboratory of the American Smelting and Refining Company at Salt Lake City, Utah, two plots of approximately

equal stand were covered with the celluloid cabinets, and their photosynthesis and respiration were measured for several days in July, 1933. Then from 1:15 to 2:00 P.M. on July 17 one of the plots, no. 4—14, was given a 45-minute fumigation. The average sulphur dioxide concentration at intake was 7.0 p.p.m. (maximum 8.6 p.p.m.) and the average outlet concentration was 4.0 p.p.m. (maximum 5.1 p.p.m.). This treatment caused about 95 per cent. acute leaf destruction. The carbon dioxide exchange data of this plot and its check are presented in figure 1. The abscissa represents time, and the ordinate represents grams of carbon dioxide absorbed in each 64-minute period. Positive values indicate photosynthesis, negative values, respiration. It will be seen that within an hour, the fumigation caused the plot to cease assimilating carbon dioxide and actually to commence evolving carbon dioxide as if the plot were in the dark. On the next two days assimilation and respiration practically balanced each other, but for a few hours each day there was a small amount of assimilation. Thereafter, with the gradual appearance of new leaves, the plot increased in ability to assimilate, until on the ninth day the activity was about 65 per cent. of the check; and, allowing for the fact that before the fumigation the plot was 15 per cent. less active than the check, the recovery on this day was about 75 per cent.

Observation of plot 4–14 indicated that on July 19, 90 per cent. to 95 per cent. of the leaf tissue had been destroyed. Some of the lower leaves in the center of the plot were uninjured. On July 21 new leaves were seen to be forming, and dry leaves were falling off. On July 26 the plots were harvested. At this time 23 per cent. of the dry weight of the plants consisted of normal green leaves, 7 per cent. of bleached leaves, and 70 per cent. of stems. In addition, 135 grams of leaves were shed, representing 10 per cent. of harvest weight. The check plot had 35 per cent. of leaves. The sum of the percentages of green, marked, and shed leaves on the treated plot was thus greater than the percentage of leaves on the check plot, indicating a much more rapid growth of leaves on the treated plot, subsequent to the fumigation, than on the check.

The decrease in total assimilation in the period following the fumigation represented 35 per cent. of the harvest weight of the check plot, making allowance for the fact that the check plot was 15 per cent. more active than the treated plot for two days before the fumigation. The harvest weights of these crops, which grew from June 16 to July 26, were 1530 grams for the check and 1300 grams (plus 135 grams of dead leaves which had been shed) for the treated plot. The observed decrease in yield of 15 per cent. was only about half the decrease in net assimilation because, at this stage of growth, less than half of the assimilated carbon dioxide was utilized for top growth, the remainder probably going into the roots.

The experiment described above was the first that was carried out with two units of the carbon dioxide apparatus. The results obtained, while con-

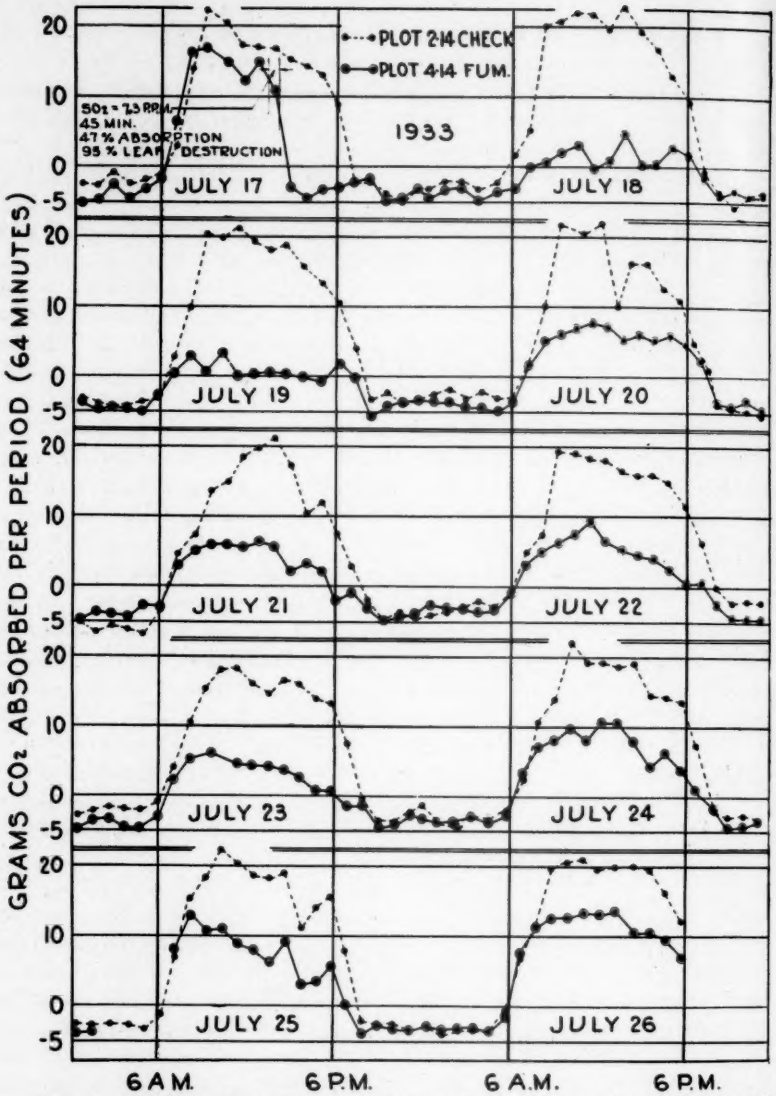

FIG. 1. Apparent assimilation and respiration of plots 2–14 and 4–14 (1933). Plot 4–14 was almost completely defoliated on July 17 by a sulphur dioxide fumigation. The curves show the subsequent recovery of the photosynthetic functions with the growth of new leaves.

clusive, were less accurate than later experiments, owing to initial difficulties with the machines. Another similar experiment will, therefore, be referred to briefly which confirms the results obtained with plot 4–14.

At 11:45 A.M. on September 1, 1933, a plot was accidently fumigated with a concentration of 50 p.p.m. SO_2 for nearly 3 minutes, which also caused about 95 per cent. leaf destruction. The photosynthetic activity of this plot declined gradually to the compensation point over a period of 2 hours. The carbon dioxide exchange did not become negative until evening. Thereafter the behavior of the fumigated plot with respect to the check was similar to the behavior depicted in figure 1. During the 3 days following the fumigation, apparent assimilation was about 7 per cent. of the check; then the level of activity began to rise. In 10 days, the level was about 65 per cent. of the check, and in 15 days about 80 per cent. At this time the plot was harvested. The dry weight of the plants consisted of 24 per cent. of green leaves, 12 per cent. of marked leaves, and 64 per cent. of stems. The check plot had 36 per cent. of green leaves and 64 per cent. of stems.

B. EFFECT OF SHORT FUMIGATIONS WITH HIGH CONCENTRATIONS OF SULPHUR DIOXIDE.—In the remainder of the experiments described in this paper, an attempt has been made to avoid producing an appreciable amount of leaf destruction. The next experiment to be described extended from August 1 to 11, 1933. The plots were third-crop alfalfa, which had been cut for the first crop on June 12, and for the second on July 12. Fumigation data for plot 2–13, which was treated on August 4, 7, and 10, are presented in table I. The data include the time and duration of the fumigations, air volumes and sulphur dioxide analyses, quantity and rate of absorption of the gas by the plants, and the extent of leaf destruction.

Carbon dioxide exchange data for plot 2–13 and its check are presented in detail in figure 2, and in summarized form in table II. It will be noted that on August 2 and 3 the apparent assimilation of the two plots was nearly identical. The data on August 1 were incomplete. At 10:30 A.M. on August 4 plot 2–13 was fumigated for 12 minutes, the sulphur dioxide being 8.5 p.p.m. at the intake and 4.0 p.p.m. at the outlet. At the first sign of injury to the leaves the fumigation was stopped. Actually 2.9 per cent. of the leaf area was destroyed. The effect of the treatment on photosynthesis was immediate, and during the hour following the fumigation the photosynthetic level fell to one third of its previous value; then the rate began to increase, but it was still subnormal at the end of the day. No effect was evident on the respiration level the following night. The photosynthetic level of the treated plot was 77 per cent. of the level of the check plot on August 5, and 87 per cent. on August 6. Recovery was complete on August 7. In fact the photosynthetic activity of the treated plot exceeded that of the check about 10 per cent. on both August 7 and 8.

TABLE I

FUMIGATION DATA OF PLOT 2–13—1933

DATE (1933)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO_2		SO_2 ABSORBED			LEAF DESTRUCTION (PERCENTAGE OF TOTAL LEAF AREA)
	STARTED	DURATION		INTAKE	OUTLET				
		min.	*l.p.m.*	*p.p.m.*	*p.p.m.*	*%*	*gm.*	*rate**	*%*
Aug. 4	10:30 A.M.	12	3300	8.5	4.0	53	0.40	85.0	2.9
" 7	4:50 P.M.	78	4300	1.0	0.7	30	0.22	6.6	0.0
" 10	11:00 A.M.	70	4250	1.9	1.0	47	0.59	19.0	14.0
Total weight of SO_2 absorbed (grams)									1.21
Total weight of S absorbed (grams)									0.605

* Milligrams SO_2 absorbed per minute per 1000 gm. dry leaves. Allowance made for 10 per cent. absorption by cabinet and soil, and for 5 per cent. absorption by stems, in all calculations of "rate of SO_2 absorption" in tables I, III, VI, IX, XII, XVI, XIX, and XXIII.

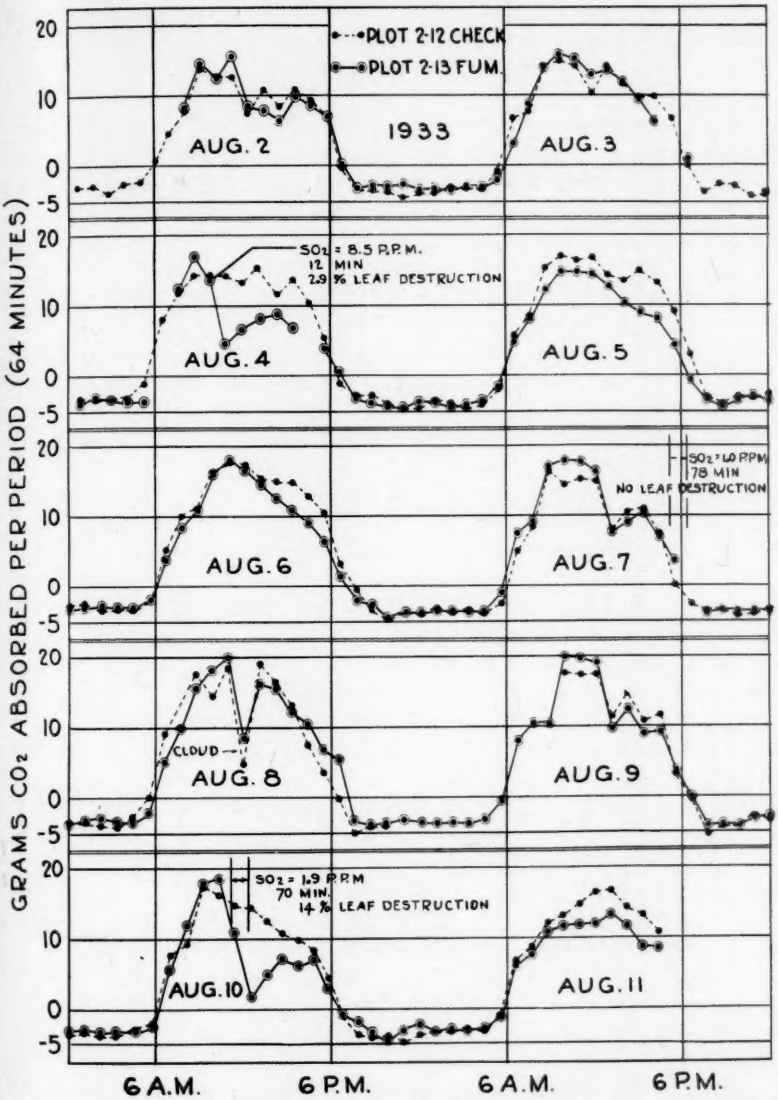

FIG. 2. Apparent assimilation and respiration of plots 2–12 and 2–13 (1933). Plot 2–13 was fumigated on August 4, 7, and 10 with high concentrations of sulphur dioxide but without causing extensive leaf destruction. The effect of the treatments on subsequent photosynthesis is shown.

TABLE II

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA
PLOTS 2–12 AND 2–13—1933

DATE (1933)	TOTAL APPARENT ASSIMILATION CO_2			NIGHT RESPIRATION CO_2			NET ASSIMILATION CO_2		
	PLOT 2–12 CHECK	PLOT 2–13 FUMIGATED	RATIO 2–13/2–12	PLOT 2–12 CHECK	PLOT 2–13 FUMIGATED	RATIO 2–13/2–12	PLOT 2–12 CHECK	PLOT 2–13 FUMIGATED	RATIO 2–13/2–12
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
Aug. 1	40.0	36.6	92	27.9	28.3	102	12.1	8.3	69
" 2	106.5	106.2	100	32.5	29.0	89	74.0	77.2	104
" 3	119.7	117.9	98	31.3	33.1	106	88.4	84.8	96
" 4			109[a]						
" 4			32[b]						
" 4			53[c]						
" 4	133.8	96.5	72[d]	38.8	37.9	98	95.0	58.6	62
" 5	147.5	113.1	77	31.7	33.3	105	115.8	79.8	69
" 6	147.0	127.5	87	32.8	32.3	99	114.2	95.2	83
" 7	110.1	121.6	110[e]	36.8	36.6	99	73.3	85.0	116
" 8	131.1	142.1	108	36.9	33.3	90	94.2	108.8	116
" 9	128.1	127.9	100	35.7	32.0	90	92.4	95.9	104
" 10			107[a]						
" 10			12[b]						
" 10			62[c]						
" 10	125.9	95.3	76[d]	33.5	28.2	84	92.4	67.1	73
" 11	126.9	102.4	81				126.9	102.4	81
Totals	1317	1187	90	338	324	96	979	863	88

Equivalent dry matter (grams) (44 per cent. carbon)	606	535
Dry matter August 11 (grams at harvest)	1145	1055
Dry matter August 1 (estimated from weight of equivalent plot harvested August 1) (grams)	665	665
Top growth during experiment (grams)	480	390
Top growth—percentage of total CO_2 assimilated	79	73
Probable root increment (grams)	126	145

[a] Before fumigation. [b] During fumigation. [c] After fumigation. [d] Total for day. [e] Fumigated at 5 P.M.

A 78-minute fumigation with 1.0 p.p.m. of sulphur dioxide at the intake and 0.7 p.p.m. at the outlet, commencing at 4:50 P.M. on August 7, caused no leaf destruction and did not influence measurably the course of the carbon dioxide curves.

Beginning at 11 A.M. on August 10 a 70-minute fumigation, with 1.9 p.p.m. SO_2 at intake and 1.0 p.p.m. at outlet, was applied, destroying 14 per cent. of the leaf area. This treatment was somewhat more drastic than the August 4 fumigation. Table I indicates the absorption of 0.59 gm. of SO_2 as compared with 0.40 gm. on August 4, though the rate of absorption of the latter is much more rapid than the former. Apparent assimilation fell at once to 12 per cent. of its former value, and then began to rise in the second hour after the treatment. The level on August 11 was 81 per cent. of the check, but the experiment was unfortunately stopped before recovery from the treatment was complete. The curves on August 10 and 11 were quite similar to those on August 4 and 5, and it is probable that complete recovery would have occurred in about the same way as before, if the plots had not been harvested so soon, though the leaf destruction on August 10 would have delayed recovery slightly. Similar effects have been observed in a number of other experiments, and it appears certain that fumigations of the order of severity indicated, cause immediate interference with photosynthesis, followed by a rapid recovery of the function if only a small amount of leaf destruction has taken place.

The decrease in net assimilation due to the fumigations represented 6.2 per cent. of the yield of the check plot. Since only 76 per cent. of the net assimilation was converted into top growth, the calculated decrease in the latter due to the fumigations was 4.7 per cent. These values are somewhat low because recovery of the treated plot was not complete when the experiment was discontinued. They afford satisfactory confirmation, within the experimental errors involved, of the equation in an earlier paper (**2**, p. 234, equation 2) for two fumigations of a single crop producing an average of 8.5 per cent. leaf destruction. Equation 2 indicates a reduction in yield of 8.7 per cent., or if the origin of equation 2 is shifted to 100 per cent. the reduction would be 4.2 per cent.

Chemical analyses of representative samples of the two plots before and after the experiment showed an increment in sulphur content of the plants, 0.55 gm. greater in the case of the treated plot than the check, as compared with 0.605 gm. of sulphur added to the treated plot as shown by the gas analyses in table I. The 9 per cent. difference probably represented absorption of sulphur dioxide by the walls of the plant chamber and the soil.

II. Experiments at Logan, Utah

Preliminary experiments with concentrations of less than 1 p.p.m. were carried out at the Salt Lake City laboratory in 1933 and 1934. In order to

Probable root increment (grams) 120

[a] Before fumigation. [b] During fumigation. [c] After fumigation. [d] Total for day. [e] Fumigated at 5 P.M.

TABLE III

FUMIGATION DATA OF PLOT 4—1936

DATE (1936)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO_2		SO_2 ABSORBED			LEAF DESTRUCTION	
	STARTED	DURATION		INTAKE	OUTLET				ACUTE	CHLOROTIC
		min.	*l.p.m.*	*p.p.m.*	*p.p.m.*	%	*gm.*	*rate**	%	%
July 21	9:26 A.M.	170	3250	0.690	0.133	80.7	0.677	7.7	0.1	
" 23	8:47 A.M.	159	3680	0.656	0.141	78.5	0.665	8.1	0.1	
" 25	10:08 A.M.	64	4125	0.811	0.140	82.7	0.390	11.0	Traces	2.0
" 28	12:35 P.M.	60	4130	0.840	0.192	77.2	0.354	10.2	Traces	
" 29	11:16 A.M.	18	4200	0.900	0.171	81.0	0.121	11.0	0.5	5.0
" 31	10:11 A.M.	14	5820	1.26	.....	.....	0.140[a]	.....	.....	
August 3	7:00 P.M.	253	6050	0.978	0.826	15.5	0.511	3.0	.....	
" 5[b]									12.0	14.0
" 8[c]									3.0	11.0

Duration of fumigations (hours) 12.3
Total weight of SO_2 absorbed (grams) 2.86
Total weight of S absorbed (grams) 1.43

* Milligrams SO_2 absorbed per minute per 1000 gm. dry leaves.

[a] Estimated.

[b] From small sample.

[c] From entire harvest. Three per cent. weather discolorations on plot 3.

develop this phase of the problem in a place where sulphur dioxide would always be absent from the air, arrangements were made to work at the Agricultural College at Logan, Utah, during the seasons of 1935 and 1936. Nine 20 by 20-foot plantings of one-year-old alfalfa, belonging to the Botany Department of the College, were available. The areas represented a number of varieties of alfalfa. All exhibited vigorous growth and were uniform in stand. It was possible to place two plant chambers on an area, one to be treated, the other to serve as a check, and to have additional comparable material growing outside the plant chambers under entirely natural conditions.

C. Effect of a series of fumigations with 0.7 to 1.26 p.p.m. SO_2.—Plots no. 3 and no. 4 were studied from July 15 to August 6, 1936. The alfalfa was Turkestan Selection no. 19,301. The fumigation data for plot 4 are presented in table III, which gives the time and duration of each treatment, together with the concentrations of sulphur dioxide applied and the amount and rate of absorption of the sulphur dioxide by the vegetation. The effect of these treatments, as measured by the carbon dioxide exchange, is shown in figure 3, charts A and B, and also in table IV, which summarizes the relations of the fumigated and check plots, and gives the relative values of the photosynthetic levels before, during, and after each fumigation.

A fumigation of 2.83 hours, with 0.69 p.p.m. on the morning of July 21 caused the photosynthetic activity to fall 18 per cent. during the time the gas was applied, but one hour after the fumigation was discontinued the activity was normal. A similar result was obtained on July 23. On July 25 a fumigation of 1.07 hours, with 0.81 p.p.m. at 11 a.m., caused the photosynthetic activity to fall about 44 per cent. during the hour that the treatment was applied. About an hour thereafter the plot came back completely to normal. A similar treatment at noon on July 28 caused a lowering of photosynthesis of about 25 per cent. The afternoon hours following this treatment were very cloudy, and the photosynthetic levels of the two plots were too low for differences to be significant. On the 29th, a fumigation of 18 minutes with 0.90 p.p.m. of sulphur dioxide lowered the photosynthetic rate about 13 per cent. for one hour and thereafter the plot returned to normal. A similar result was obtained on July 31, with 14-minutes application of 1.26 p.p.m. None of these treatments produced more than 1.0 per cent. of leaf destruction.

In the evening of August 3, a 4-hour fumigation with 0.98 p.p.m. was applied from 7 to 11:13 p.m. While the gas was present, the rate of respiration of the treated plot was only 62 per cent. of the check. During the remainder of the night the ratio was 120 per cent., which was about the normal ratio for the respiration of plots 3 and 4. Respiration appeared

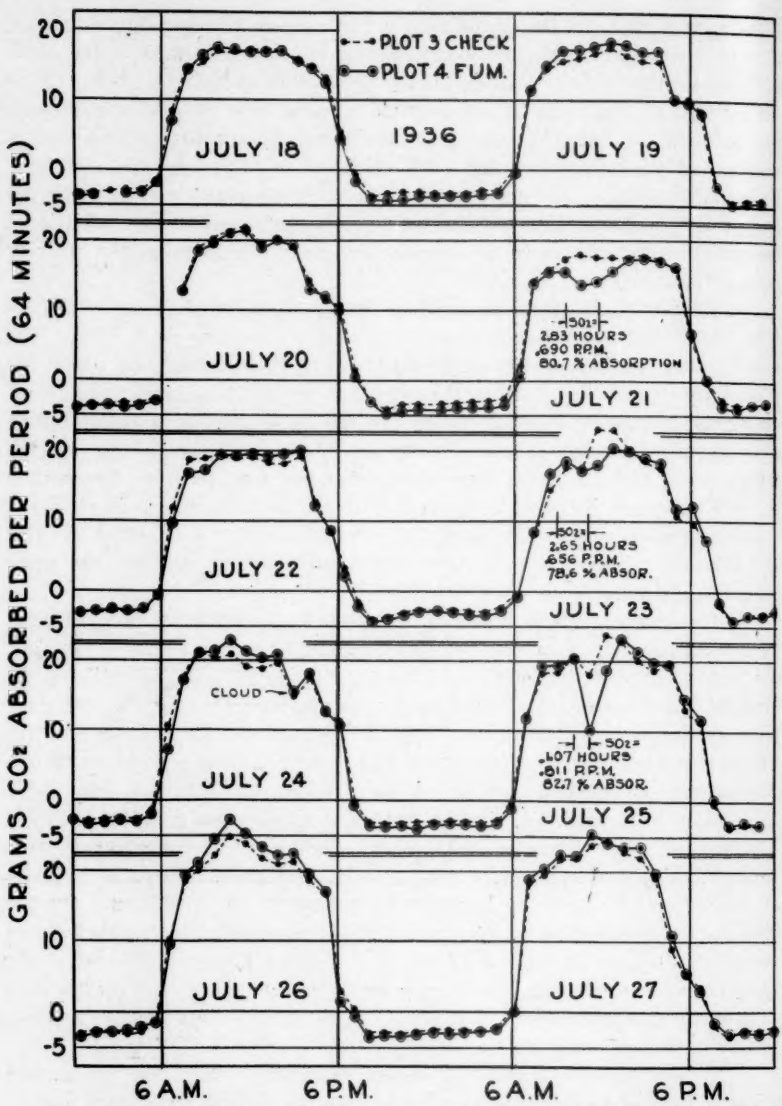

FIG. 3. Chart A, July 18–27, 1936. Apparent assimilation and respiration of plots 3 and 4 at Logan, Utah. The curves show the depressing effect on photosynthesis during the treatment of plot 4 of seven short fumigations with 0.7 to 1.26 p.p.m. SO_2, together with the rapid recovery of a normal or greater than normal photosynthetic level when the treatment was discontinued.

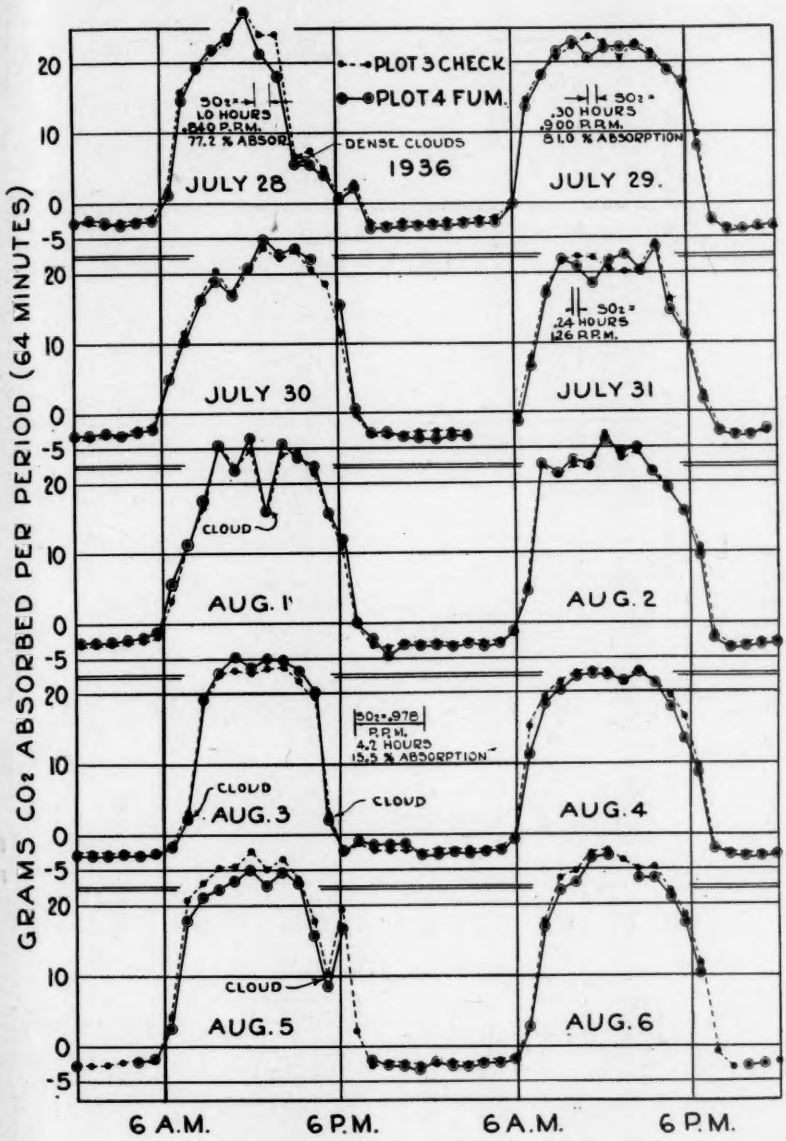

FIG. 3. Chart B, July 28–August 6, 1936.

TABLE IV

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA
PLOTS 3 AND 4—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO_2			NIGHT RESPIRATION CO_2			NET ASSIMILATION CO_2		
	PLOT 3 CHECK	PLOT 4 FUMIGATED	RATIO 4/3	PLOT 3 CHECK	PLOT 4 FUMIGATED	RATIO 4/3	PLOT 3 CHECK	PLOT 4 FUMIGATED	RATIO 4/3
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
July 15–20*	926.9	928.4	100	182.8	212.5	116	744.1	715.9	96
July 21			99[a]						
" "			82[b]						
" "			100[c]						
" "	179.4	165.6	92[d]	27.2	28.4	104	152.2	137.2	90
July 22*	187.3	184.2	98	29.0	32.4	112	158.3	151.8	96
July 23			111[a]						
" "			92[b]						
" "			105[c]						
" "	189.4	189.0	100[d]	27.7	29.8	108	161.7	159.2	98
July 24*	203.4	208.4	102	28.9	33.8	117	174.5	174.6	100
July 25			104[a]						
" "			56[b]						
" "			104[c]						
" "	216.4	209.4	97[d]	27.7	26.7	96	188.7	182.7	97
July 26–27*	429.9	449.1	104	52.6	56.0	106	377.3	393.1	104
July 28			100[a]						
" "			75[b]						
" "			80[c]						
" "	179.4	164.6	92[d]	23.2	28.5	123	156.2	136.1	87
July 29			101[a]						
" "			87[b]						
" "			100[c]						
" "	234.5	229.7	98[d]	30.8	30.5	99	203.7	199.2	98

TABLE IV—(*Continued*)

Date (1936)	Total apparent assimilation CO_2			Night respiration CO_2			Net assimilation CO_2		
	Plot 3 check	Plot 4 fumigated	Ratio 4/3	Plot 3 check	Plot 4 fumigated	Ratio 4/3	Plot 3 check	Plot 4 fumigated	Ratio 4/3
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
July 30*	210.4	216.8	103	24.0	27.4	114	186.4	189.4	102
July 31			96[a]						
" "			83[b]						
" "			101[c]						
" "	207.5	201.4	97[d]	28.4	26.0	92	179.1	175.4	98
August 1–2*	453.2	461.3	102	55.4	60.2	109	397.8	401.1	101
August 3						62[b]			
" "						120[c]			
" "	183.2	190.1	104	22.9	22.2	97[d]	160.3	167.9	105
August 4–5*	492.7	452.5	92	47.7	50.7	106	445.0	401.8	90
August 6–7*	513.4	496.3	97	22.8	23.5	103	490.6	472.8	96
Totals	4807.0	4747.0	99	631.0	689.0	109	4176.0	4058.0	97

Equivalent dry matter (grams) (44 per cent. carbon)	2590	2520
Dry matter August 6 (grams at harvest)	2330	2279
Dry matter July 15 (estimated from weight of equivalent plot harvested July 15) (grams)	852	870
Top growth during experiment (grams)	1478	1409
Top growth—percentage of total CO_2 assimilated	57	56
Probable root increment (grams)	1112	1111

* No fumigation on these days.
[a] Before fumigation.
[b] During fumigation.
[c] After fumigation.
[d] Total for the day.

to be depressed during the treatment, but became normal soon afterward. Conspicuous acute markings developed the next day. Acute leaf destruction, measured from a small sample on August 5, was 12 per cent. of the total leaf area, and chlorotic markings were 14 per cent. At harvest on August 8, these values were 3 per cent. and 11 per cent., respectively. The rate of apparent assimilation was reduced 8 per cent. on August 4 and 5, and 3 per cent. on August 6 and 7, showing that interference with photosynthesis paralleled leaf destruction, and showing also that recovery was practically complete in 4 days.

Table IV shows that the apparent assimilation values of the two plots, which were equal during the pre-fumigation period from July 15 to July 20, were not significantly different over the whole period of the experiments, although measurable reductions in the rate of apparent assimilation occurred during each of the fumigations. Table IV and figure 3 indicate a tendency for the photosynthetic level of the treated plot to rise above the level of the check plot during part of the afternoon following the treatment, and also on the following day, suggesting a compensating stimulation immediately following the fumigation. This tendency, though slight, is sufficient to offset practically all of the reductions due to the fumigations. The harvest weights of the two crops were not significantly different.

A number of complete shoots were removed from the plots at intervals for chemical analyses. These shoots were taken at random from all parts of the plot through the 5 sample windows with which the fumigation cabinets were provided. All the samples were taken as nearly as possible at the same time of day so that they would be comparable in carbohydrate content. The leaves were immediately separated from the stems. When the plots were finally harvested, equal portions from each plot were cut simultaneously to avoid differences in carbohydrate content of the leaves, and a complete separation of leaves and stems was made. Portions of these samples were analyzed immediately for chlorophyll by the method of WILLSTÄTTER and STOLL, modified by SCHERTZ (3), using GUTHRIE'S (1) ammoniacal copper sulphate-potassium dichromate solution as the working reference. The latter was standardized against preparations from known amounts of pure pigment. Chlorophyll data for leaves and stems are given in table V. They indicate that the fumigated plot remained practically constant in chlorophyll content throughout the period of the experiment, whereas the check plot increased a little in this constituent, while the plants growing outside of the plant chamber decreased slightly in chlorophyll. Evidently the slightly reduced amount of chlorophyll in the treated leaves as compared with the check leaves did not impair measurably their photosynthetic activity. Variations of chlorophyll in the stems are without significance.

TABLE V

ANALYSES OF PLOTS 3 AND 4 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 3 (CHECK)			PLOT 4 (FUMIGATED)			PLOT 3 CHECK	PLOT 4 FUMIGATED	OUTSIDE* CHECK
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)			
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
July 15	Leaves	.....	.....	.....	.....	.....	.....	.....	.....	1.69
" 20	"	.....	.....	.....	.....	.....	.....	1.80	1.73	.....
" 26	"	.....	.....	.....	.....	.....	.....	1.81	1.57	1.60
" 29	"	.....	.....	.....	.....	.....	.....	2.02	1.60	.....
August 8	Normal leaves	606	0.503	3.05	395	0.814	3.22	1.97	1.68	1.53
" 8	Marked "	.....	.....	.....	225	0.762	1.71	.....	1.42	.....
July 20	Stems	.....	.....	.....	.....	.....	.....	0.21	0.24	.....
" 26	"	.....	.....	.....	.....	.....	.....	0.22	0.26	0.22
" 29	"	.....	.....	.....	.....	.....	.....	0.24	0.23	.....
August 8	"	1570	0.162	2.54	1515	0.197	2.98	0.27	0.29	0.19
" 8	Dead tissue	120	0.468	0.56	115	0.445	0.51	.....	.....	.....
" 8	Leaves and stems	.....	0.257	.....	.....	0.371	.....	.....	.....	.....
	Total			6.15			8.42			
	Excess of sulphur in plot 4 over plot 3						2.27			

* Comparable alfalfa grown adjacent to and outside of plant chambers.

TABLE VI

FUMIGATION DATA OF PLOT 12—1936

DATE (1936)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO_2		SO_2 ABSORBED			LEAF DESTRUCTION	
	STARTED	DURATION		INTAKE	OUTLET				ACUTE	CHLOROTIC
		min.	*l.p.m.*	*p.p.m.*	*p.p.m.*	%	*gm.*	*rate**	%	%
August 8	9:30 A.M.	254	5530	0.579	0.424	26.8	0.48	8.1	.....	.....
" 9	9:22 A.M.	254	5520	0.574	0.423	26.3	0.47	7.5	.....	.....
" 10	8:59 A.M.	252	5470	0.567	0.412	27.2	0.47	7.2	Traces	1.0
Av. Aug. 8–10	.....	253	5510	0.573	0.420	26.8	0.473	.....	Traces	.....
August 15	8:56 A.M.	240	5500	0.603	0.460	23.7	0.42	5.5	.....	.....
" 16	8:59 A.M.	240	5390	0.622	0.452	27.3	0.49	6.2	0.6	3.0
" 17	8:57 A.M.	240	5470	0.657	0.382	41.9	0.80	9.8	.....	.....
Av. Aug. 15–17	.....	240	5450	0.627	0.431	31.0	0.57	.....	0.2	2.3
August 21[a]										

Duration of fumigations (hours)	24.7
Total weight of SO_2 absorbed (grams)	3.13
Total weight of S absorbed (grams)	1.57

* Milligrams SO_2 absorbed per minute per 1000 gm. dry leaves.

[a] 0.9 per cent. weather discolorations on check (plot 11).

Analyses of the leaves and the stems for total sulphur at harvest are also given in table V. It will be noted that the sulphur content of the fumigated leaves was about 0.8 per cent. as compared with 0.5 per cent. in the check leaves. The fumigated stems were also somewhat higher in sulphur than the check stems. When the total amount of sulphur in the system was calculated, it was found that there was an excess of 2.27 gm. of sulphur in plot 4 over plot 3. This is appreciably greater than the total weight of sulphur absorbed, 1.43 gm., as indicated by the gas analyses in table III. This discrepancy can be ascribed to the fact that plot 4 had been given an extensive fumigation in 1935, described below, which had enriched the roots and permitted additional sulphur nutrition on subsequent crops. This point will be referred to again.

D. Effect of fumigations with approximately 0.6 p.p.m.—Plots 11 and 12 of third-crop alfalfa were observed from August 5 to August 20, 1936. This variety of alfalfa is Province no. 34886. The alfalfa had been previously cut on June 26 and July 27. The fumigation data of plot 12 are presented in table VI. Plot 12 received 3 fumigations, each of 4.2 hours duration, on the mornings of August 8, 9, and 10. The average intake concentration was 0.573 p.p.m., the outlet 0.420 p.p.m., and the mean 0.497 p.p.m. Then following a rest period of 4 days, plot 12 was again fumigated on 3 successive mornings with 3 fumigations, each of 4.0 hours duration, with an intake concentration of 0.627 p.p.m., the outlet 0.433 p.p.m., and the mean 0.530 p.p.m. Both acute and chlorotic markings could be seen scattered generally over the plot. When the actual leaf areas destroyed were measured at harvest time, the amounts were 2.5 per cent. One small plant, stunted by bacterial wilt, had 50 per cent. acute markings.

Curves of photosynthetic activity and respiration of plots 11 and 12 are presented in figure 4, charts A and B, and the carbon dioxide exchange data are summarized and compared in table VII. The treatments in every case reduced the photosynthetic activity of the treated plot during the time that the fumigation was in progress, but within 2 or 3 hours after the fumigation was discontinued the plot was practically normal again in its activity. The average rate of assimilation on plot 12 during the progress of the first 3 fumigations was 76 per cent. of the check, and 74 per cent. during the second 3 fumigations. Considering the 4 hours of the afternoons on these days, starting in each case about an hour after the fumigation had been discontinued, the average rate of photosynthesis on the treated plot was 98 per cent. of the check in the first 3 fumigations, and 88 per cent. in the second 3 fumigations. During the 4-day rest period between the two series of fumigations, the rate of photosynthesis of plot 12 was 98 per cent. of plot 11, and during 3 days following the last treatment, 93 per cent. of the check.

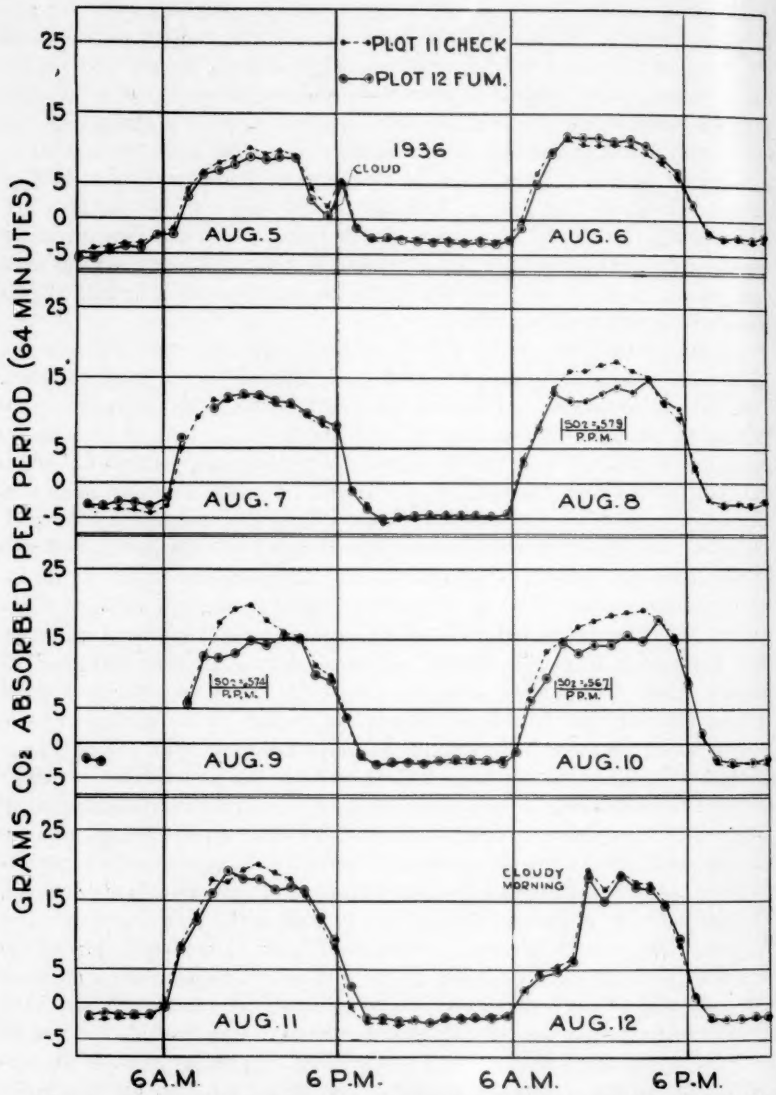

FIG. 4. Chart A, August 5–12, 1936. Apparent assimilation and respiration of plots 11 and 12 at Logan, Utah. Plot 12 received six 4-hour fumigations with about 0.6 p.p.m. SO_2 on six mornings. The curves show that photosynthesis on plot 12 is depressed during the treatment, but a rapid recovery occurs when the treatment is discontinued.

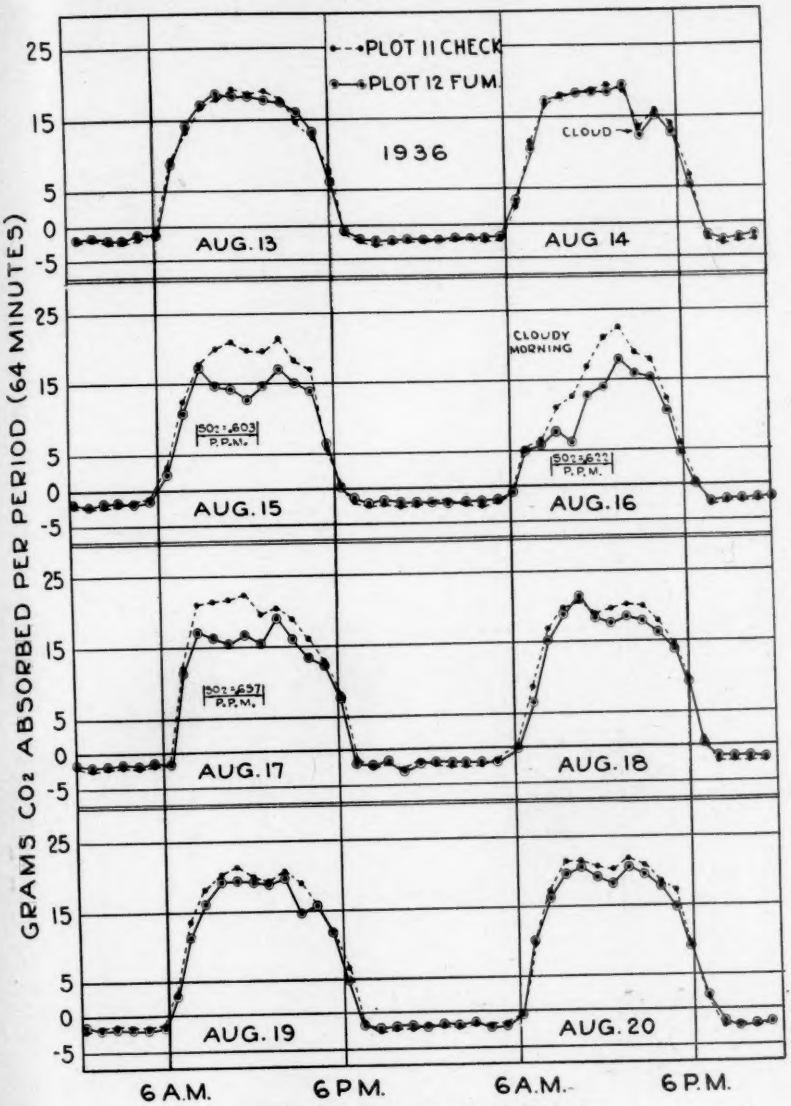

FIG. 4. Chart B, August 13–20, 1936.

TABLE VII

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA
PLOTS 11 AND 12—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO_2			NIGHT RESPIRATION CO_2			NET ASSIMILATION CO_2		
	PLOT 11 CHECK	PLOT 12 FUMIGATED	RATIO 12/11	PLOT 11 CHECK	PLOT 12 FUMIGATED	RATIO 12/11	PLOT 11 CHECK	PLOT 12 FUMIGATED	RATIO 12/11
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
August 5– 7	279.3	277.4	99	91.7	88.3	96	187.6	189.1	101
August 8–10	.....	.....	95[a]	.....	.....	.....	.....	.....	.....
"	.....	.....	76[b]	.....	.....	.....	.....	.....	.....
"	.....	.....	98[c]	.....	.....	.....	.....	.....	.....
"	472.1	403.9	86[d]	73.7	70.5	96	398.4	333.4	84
August 11–14	642.7	627.8	98	92.7	81.2	88	550.0	546.6	99
August 15–17	.....	.....	92[a]	.....	.....	.....	.....	.....	.....
"	.....	.....	74[b]	.....	.....	.....	.....	.....	.....
"	.....	.....	88[c]	.....	.....	.....	.....	.....	.....
"	524.1	417.9	80[d]	65.7	59.0	90	458.4	358.9	78
August 18–20	576.0	533.2	93	52.2	47.5	91	523.8	485.7	93
Totals	2494	2260	91	376	347	92	2118	1914	90

Equivalent dry matter (grams) (44 per cent. carbon)	1311	1187	
Dry matter August 21 (grams at harvest)	1123	966	86%
Dry matter August 5 (estimated from weight of equivalent plot harvested August 5) (grams)	340	320	
Top growth during experiment (grams)	783	646	
Top growth—percentage of total CO_2 assimilated	58.8%	54.5%	
Probable root increment (grams)	528	541	

[a] Before fumigation. [b] During fumigation. [c] After fumigation. [d] Total for the period.

TABLE VIII

ANALYSES OF PLOTS 11 AND 12 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 11 (CHECK)			PLOT 12 (FUMIGATED)			PLOT 11 CHECK	PLOT 12 FUMIGATED	OUTSIDE* CHECK
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)			
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
August 5	Leaves	.....	.....	.....	.....	.....	.....	.....	.....	1.25
" 9	"	.....	.....	.....	.....	.....	.....	1.58	1.54	1.50
" 12	"	.....	.....	.....	.....	.....	.....	1.64	1.50	1.47
" 14	"	11.3	0.542	0.061	9.1	0.851	0.077	1.76	1.63	1.71
" 17	"	19.8	0.585	0.116	15.3	1.13	0.173	1.62	1.53	.....
" 22	Upper leaves	315.0	0.600	1.890	264.0	1.050	2.772	1.73	1.63	1.42
" 22	Lower "	71.0	0.685	0.486	75.0	1.273	0.955	1.86	1.66	1.62
August 5	Stems	.....	.....	.....	.....	.....	.....	.....	.....	0.35
" 9	"	.....	.....	.....	.....	.....	.....	0.38	0.32	0.29
" 12	"	.....	.....	.....	.....	.....	.....	0.35	0.36	0.30
" 14	"	15.3	0.226	0.035	11.2	0.265	0.030	0.35	0.36	.....
" 17	"	26.2	0.207	0.054	21.6	0.275	0.059	0.36	0.33	.....
" 22	"	615.0	0.199	1.222	538.0	0.225	1.210	0.34	0.34	0.33
" 22	Dead tissue	34.0	0.450	0.153	22.0	0.621	0.137	.....	.....	.....
" 22	Leaves and stems	.....	0.360	.....	.....	0.563	.....	.....	.....	.....
	Total			4.017			5.413			
	Excess of sulphur in plot 12 over plot 11						1.396			

* Comparable alfalfa grown adjacent to and outside of plant chambers.

The reduction in the amount of net assimilation which can be ascribed to the first 3 treatments was 3 per cent. of the total net assimilation. The last 3 treatments increased this reduction to 10 per cent. The period of observation in these experiments was only 16 days. As the normal growth period of a crop of alfalfa is at least 30 to 36 days, these percentages should be reduced to about 1.5 and 5 per cent. respectively. The harvest weight of plot 12 was 86 per cent. of the yield of plot 11. This reduced yield was due in part to the fumigation treatments and in part to the fact that the density of the stand on the treated plot was less than on the check. This was indicated by the appearance of the plots, since plot 12 had a somewhat less complete cover than plot 11, and also by the fact that there were 5 per cent. fewer average sized stems on plot 12 than on plot 11.

Chlorophyll and sulphur determinations on plots 11 and 12, similar to those described above for plots 3 and 4, are given in table VIII. Again, the check plot increased in chlorophyll content during the progress of the experiment a little more rapidly than the treated plot, but the differences are without practical significance.

The sulphur analyses of the leaves and stems show an increment of 1.39 gm. of sulphur in plot 12, which may be ascribed to the fumigation. This may be compared with a value of 1.57 gm. given by the air analyses in table VI. As the air analyses were not corrected for absorption by the fumigation cabinet or the soil, the agreement between the two values is quite satisfactory. Neither of these plots had been fumigated previously.

E. Effect of about 0.4 p.p.m.—Plots 9 and 10 were investigated from August 8 to 31, 1936. This variety of alfalfa is Turkestan no. 19315. The fumigation data for plot 9 are given in table IX. Plot 9 received 5 fumigations, each of 4.2 hours duration, on 5 successive mornings, beginning between 8 and 10 A.M., the average concentrations being 0.417 p.p.m. intake, 0.231 p.p.m outlet, and 0.324 p.p.m. mean. Plot 10 was a check. Then following a rest period of 5 days, plot 9 was given another series of 5 fumigations each of 4.2 hours duration on 5 successive mornings, the average intake concentration being 0.441 p.p.m., the average outlet concentration 0.237 p.p.m., and the mean 0.339 p.p.m. These treatments possibly produced some slight acute markings and also some chlorotic markings; but at harvest time it was difficult to distinguish markings due to sulphur dioxide, and those due to other causes. There were 0.6 per cent. more discolorations on the fumigated plot than on the check plot.

Carbon dioxide exchange data for plots 9 and 10 are given in figure 5, charts A and B, and are summarized in table X. The reduction in the rate of apparent assimilation during the time each fumigation was in progress averaged about 9 per cent. during the first 5 treatments and about 14 per cent. during the second 5 treatments. It is interesting to note that the net assimilation of plot 9 was 97 per cent. of the check plot for the 3 days

TABLE IX

FUMIGATION DATA OF PLOT 9—1936

DATE (1936)	FUMIGATION		AIR VELOCITY	CONCENTRATION OF SO_2		SO_2 ABSORBED			LEAF DESTRUCTION	
	STARTED	DURATION		INTAKE	OUTLET				ACUTE	CHLOROTIC
		min.	*l.p.m.*	*p.p.m.*	*p.p.m.*	%	*gm.*	*rate**	%	%
August 13	10:03 A.M.	251	5040	0.427	0.202	52.6	0.634	9.4	.....	.....
" 14	8:41 A.M.	251	5850	0.388	0.217	44.0	0.555	7.7	.....	.....
" 15	8:44 A.M.	252	5730	0.421	0.225	46.5	0.625	8.5	.....	.....
" 16	8:57 A.M.	252	5770	0.430	0.265	38.3	0.534	6.8	.....	.....
" 17	8:40 A.M.	251	5820	0.417	0.243	41.8	0.566	6.8	Traces	Traces
Av. Aug. 13–17	.....	251	5642	0.417	0.231	44.6	0.583	.....	.....	.....
August 23[a]	9:03 A.M.	253	5500	0.440	0.239	45.7	0.619	6.0	1.1	2.6
" 24	8:44 A.M.	254	5420	0.440	0.235	46.7	0.628	6.0	.....	.....
" 25	9:04 A.M.	253	5440	0.456	0.231	49.4	0.691	6.0	.....	.....
" 26[b]	8:53 A.M.	254	5450	0.440	0.240	45.5	0.618	5.1	1.0	1.6
" 27	8:50 A.M.	253	5475	0.429	0.239	44.3	0.593	5.1	.....	.....
Av. Aug. 23–27	.....	253	5457	0.441	0.237	46.3	0.630	.....	.....	.....
September 2[c]									0.2	1.2

Duration of fumigations (hours)	42.1
Total weight of SO_2 absorbed (grams)	6.05
Total weight of S absorbed (grams)	3.02

* Milligrams SO_2 absorbed per minute per 1000 gm. dry leaves.

[a] 1.5 per cent. weather discolorations on check (plot 10).

[b] 1.5 per cent. weather discolorations on check (plot 10).

[c] 0.8 per cent. weather discolorations on check (plot 10).

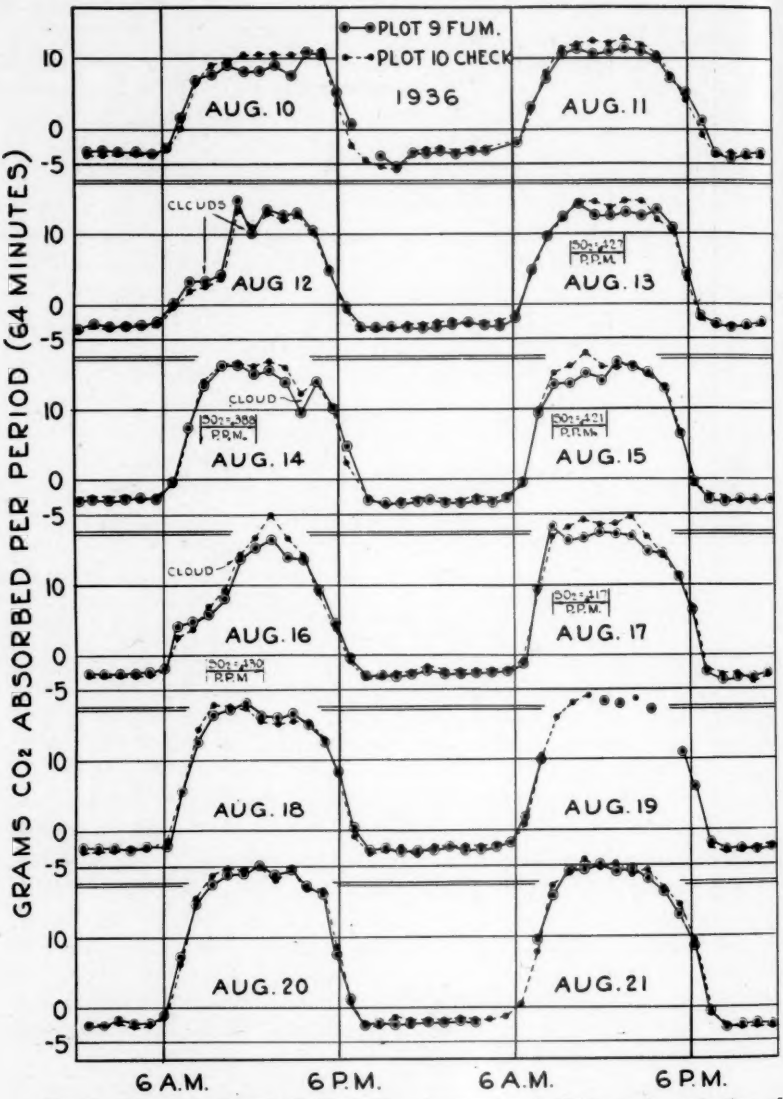

FIG. 5. Chart A, August 10–21, 1936. Apparent assimilation and respiration of plots 9 and 10 at Logan, Utah. The effects of ten 4-hour fumigations on plot 9 with 0.44 p.p.m. SO_2 on ten mornings are shown.

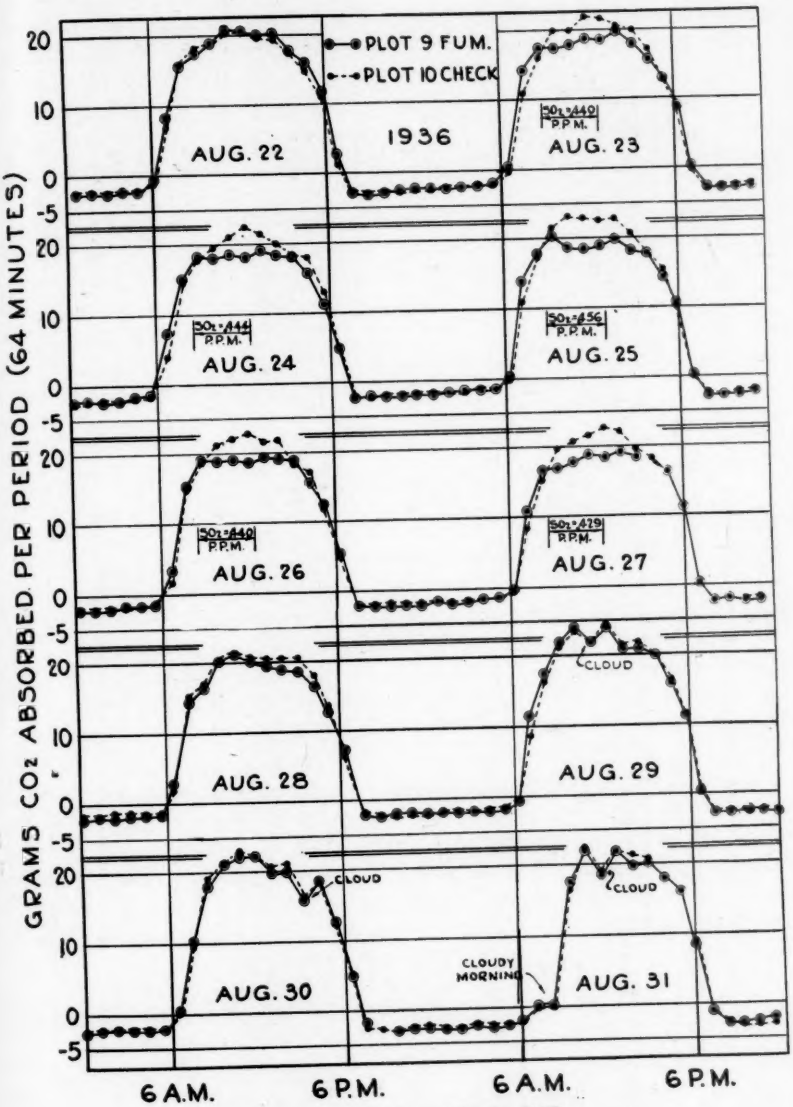

FIG. 5. Chart B, August 22–31, 1936.

TABLE X

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 9 AND 10—1936

DATE (1936)	TOTAL APPARENT ASSIMILATION CO_2			NIGHT RESPIRATION CO_2			NET ASSIMILATION CO_2		
	PLOT 10 CHECK	PLOT 9 FUMIGATED	RATIO 9/10	PLOT 10 CHECK	PLOT 9 FUMIGATED	RATIO 9/10	PLOT 10 CHECK	PLOT 9 FUMIGATED	RATIO 9/10
	gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
August 10–12	288.7	282.0	98	98.9	97.2	98	189.8	184.8	97
August 13–17	.....	.....	102[a]	.....	.....	.....	.....	.....	.....
"	.....	.....	91[b]	.....	.....	.....	.....	.....	.....
"	.....	.....	98[c]	.....	.....	.....	.....	.....	.....
"	697.0	661.7	95[d]	145.6	152.3	105	551.4	509.4	92
August 18–22	871.0	878.2	101	122.0	121.5	100	749.0	756.7	101
August 23–27	.....	.....	105[a]	.....	.....	.....	.....	.....	.....
"	.....	.....	86[b]	.....	.....	.....	.....	.....	.....
"	.....	.....	96[c]	.....	.....	.....	.....	.....	.....
"	983.0	920.7	94[d]	98.6	104.3	106	884.4	816.4	92
August 28–31	759.5	750.2	99	98.8	97.0	98	660.7	653.2	99
Totals	3599	3493	97.0	564	572	101.5	3035	2920	96.2

Equivalent dry matter (grams) (44 per cent. carbon)	1882	1810	
Dry matter September 1 (grams at harvest)	1424	1443	101
Dry matter August 10 (estimated from weight of equivalent plot harvested August 10) (grams)	485	478	
Top growth during experiment (grams)	939	965	
Top growth—percentage of total CO_2 assimilated	49.7	53.3	
Probable root increment (grams)	943	845	

[a] Before fumigation. [b] During fumigation. [c] After fumigation. [d] Total for the period.

immediately preceding the first 5 fumigations. It was 101 per cent. of the check on the 5 days succeeding these fumigations, and 99 per cent. of the check in the period following the second 5 fumigations, again suggesting a stimulation of photosynthesis by sulphur dioxide at this exposure and dilution. These increases in net assimilation largely counterbalance the reductions due to the fumigations, so that the total net assimilation of the treated plot for the period of the experiment was 96 per cent. of the check as compared with 97 per cent. in the period before fumigation treatments were started. This difference between the net assimilation values of the 2 plots is probably without significance. The 1 per cent. difference between the actual yields of the plots at harvest time in favor of the fumigated plot certainly is without significance.

Chlorophyll analyses on plots 9 and 10, given in table XI confirm the conclusions stated for the preceding plots, that there was no effect of practical significance of these fumigations on the chlorophyll.

The sulphur analyses in table XI show that there was an excess of sulphur in plot 9 over plot 10 of 2.73 gm., which can be attributed entirely to the fumigation treatments, since neither of these plots had been fumigated previously. This value compares favorably with the absorption value of 3.02 gm. determined by air analyses, as indicated in table IX. The sulphur content of fumigated leaves was increased by these fumigations to double the content of the check leaves.

F. Effect of a series of intermittent fumigations of about 0.3 p.p.m. —Plots 5 and 6 were under observation from August 24 to October 4, 1936. The variety of alfalfa was Turkestan no. 19303. This is the same pair of plots that was used in the second experiment in 1935, described later, and also in an earlier experiment in 1936. In each case, plot 6 was fumigated and plot 5 was the check. The first crop had been harvested on June 26, and the second crop on August 3. The fumigation data for plot 6 are given in table XII. Plot 6 was fumigated each morning from August 30 to September 30, inclusive, except September 1 when it was not fumigated, and except for 4 other days when it was fumigated in the afternoon instead of in the morning. The plot thus received 31 fumigations, each of 4.25 hours, totaling 132 hours. The average intake concentration was 0.348 p.p.m., the average outlet concentration 0.228 p.p.m., and the mean 0.288 p.p.m. In this experiment, in addition to observing the effect of the fumigation treatment, the plots were purposely allowed to go without irrigation water for 3 weeks in order to obesrve whether the addition of an unfavorable factor, such as drought, would have any effect upon the fumigation treatments. During the hot part of 3 days—September 17, 18, and 19—both plots wilted perceptibly, the check plot suffering somewhat more at this time than the treated plot. Thereafter they were both watered adequately. As

TABLE XI

ANALYSES OF PLOTS 9 AND 10 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 10 (CHECK)			PLOT 9 (FUMIGATED)					
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	PLOT 10 CHECK	PLOT 9 FUMIGATED	OUTSIDE* CHECK
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
August 9	Leaves	.....	.....	.....	.....	.....	.....	.....	.....	1.30
" 12	"	.....	.....	.....	.....	.....	.....	1.85	1.82	1.45
" 14	Upper leaves	8.2	0.512	0.042	8.6	0.795	0.068	1.87	1.85	1.46
" 14	Lower "	3.1	0.546	0.017	2.4	0.728	0.017	1.92	1.93	.....
" 17	Upper "	9.4	0.582	0.055	13.5	0.923	0.125	1.73	1.64	.....
" 17	Lower "	.....	.....	.....	1.6	0.805	0.013	.....	2.22	.....
" 23	Upper "	19.1	0.585	0.112	19.6	0.894	0.175	1.79	1.76	1.69
" 23	Lower "	3.2	0.575	0.018	1.2	0.863	0.010	2.04	.....	.....
" 26	Upper "	17.1	0.553	0.095	22.5	1.111	0.250	1.82	1.80	1.53
" 26	Lower "	5.5	0.601	0.033	7.8	1.068	0.083	2.15	1.81	1.76
Sept. 1	Upper "	377.0	0.518	1.953	379.0	1.013	3.840	1.75	1.68	1.52
" 1	Lower "	86.0	0.575	0.495	69.0	1.035	0.715	1.75	1.62	1.75
Aug. 9	Stems	.....	.....	.....	.....	.....	.....	.....	.....	0.37
" 12	"	.....	.....	.....	.....	.....	.....	0.28	0.29	0.30
" 14	"	15.6	0.239	0.037	16.2	0.264	0.043	0.35	0.33	.....
" 17	"	13.8	0.263	0.036	23.5	0.262	0.062	0.32	0.31	.....
" 23	"	39.8	0.186	0.074	34.3	0.221	0.076	0.29	0.31	0.31
" 26	"	38.0	0.221	0.084	52.0	0.237	0.123	0.30	0.30	.....
Sept. 1	"	750.0	0.207	1.553	736.0	0.221	1.627	0.28	0.28	0.26
" 1	Dead tissue	32.0	0.440	0.141	51.0	0.478	0.244	.....	.....	.....
" 1	Leaves and stems	.....	0.330	.....	.....	0.521	.....	.....	.....	.....
	Total			4.745			7.471			
	Excess of sulphur in plot 9 over plot 10						2.73			

* Comparable alfalfa grown adjacent to and outside of plant chambers.

TABLE XII

FUMIGATION DATA OF PLOT 6—1936

DATE (1936)	NUMBER OF FUMIGATIONS	AIR VELOCITY	DURATION EACH FUMIGATION	SO_2 CONCENTRATION EACH FUMIGATION		SO_2 ABSORBED EACH FUMIGATION			TOTAL S
				INTAKE	OUTLET				
		l.p.m.	*min.*	*p.p.m.*	*p.p.m.*	%	*gm.*	*rate**	*gm.*
August 30		Maximum[a] 5750	260	0.397	0.306	41.1	0.445	3.1	.....
to	5	Minimum[a] 5550	252	0.335	0.197	22.4	0.289	2.0	.....
September 4		Average 5660	255	0.366	0.245	33.2	0.388	2.7	0.86[b]
September 5		Maximum[a] 5660	264	0.368	0.260	45.1	0.450	3.0	.....
to	26	Minimum[a] 5240	253	0.308	0.172	26.7	0.282	1.9	.....
September 30		Average 5520	255	0.344	0.224	34.9	0.380	2.6	5.05[b]
Totals	31		7913						5.91

* Milligrams SO_2 absorbed per minute per 1000 gm. dry leaves.

[a] The maximum and minimum values in the horizontal lines of this table occurred in different fumigations and are, therefore, unrelated.

[b] Absorption of September 4 distributed equally between these totals.

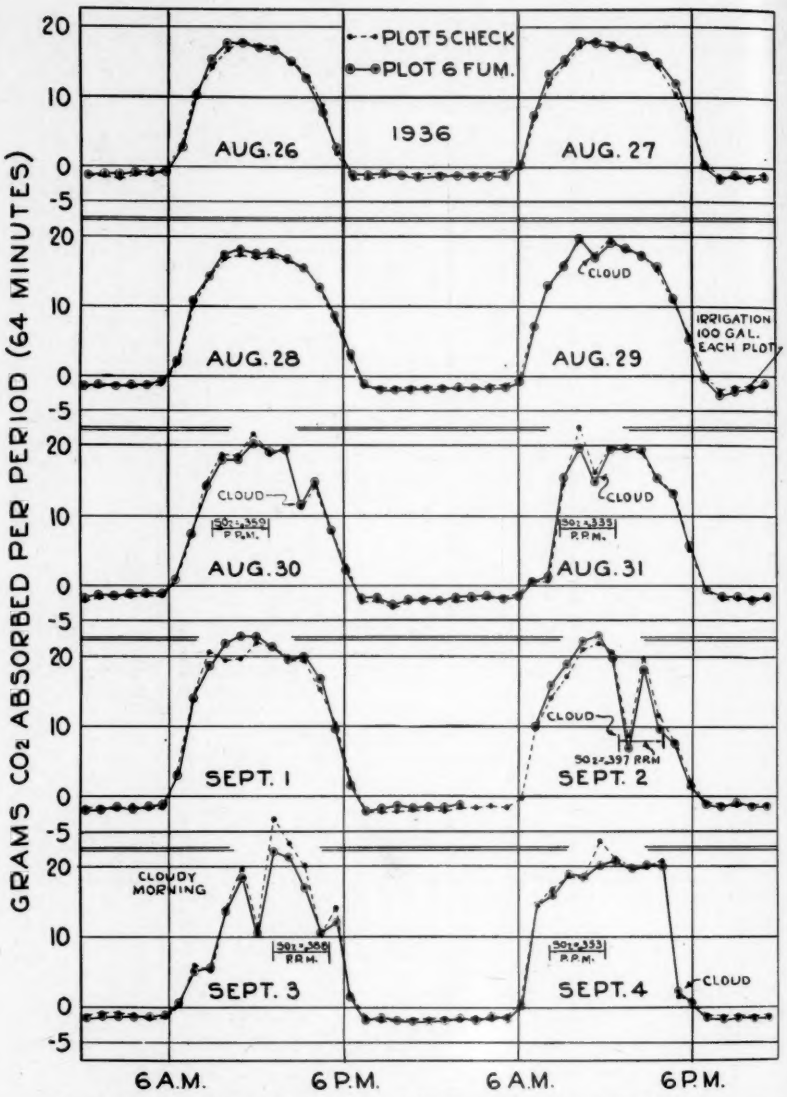

FIG. 6. Chart A, August 26–September 4, 1936. Apparent assimilation and respiration of plots 5 and 6 at Logan, Utah. Plot 6 received 31 4-hour fumigations on 27 mornings and 4 afternoons, with about 0.35 p.p.m. SO_2. In addition, water was purposely withheld from both plots from August 29 until September 19. Some temporary wilting occurred on September 17, 18, and 19.

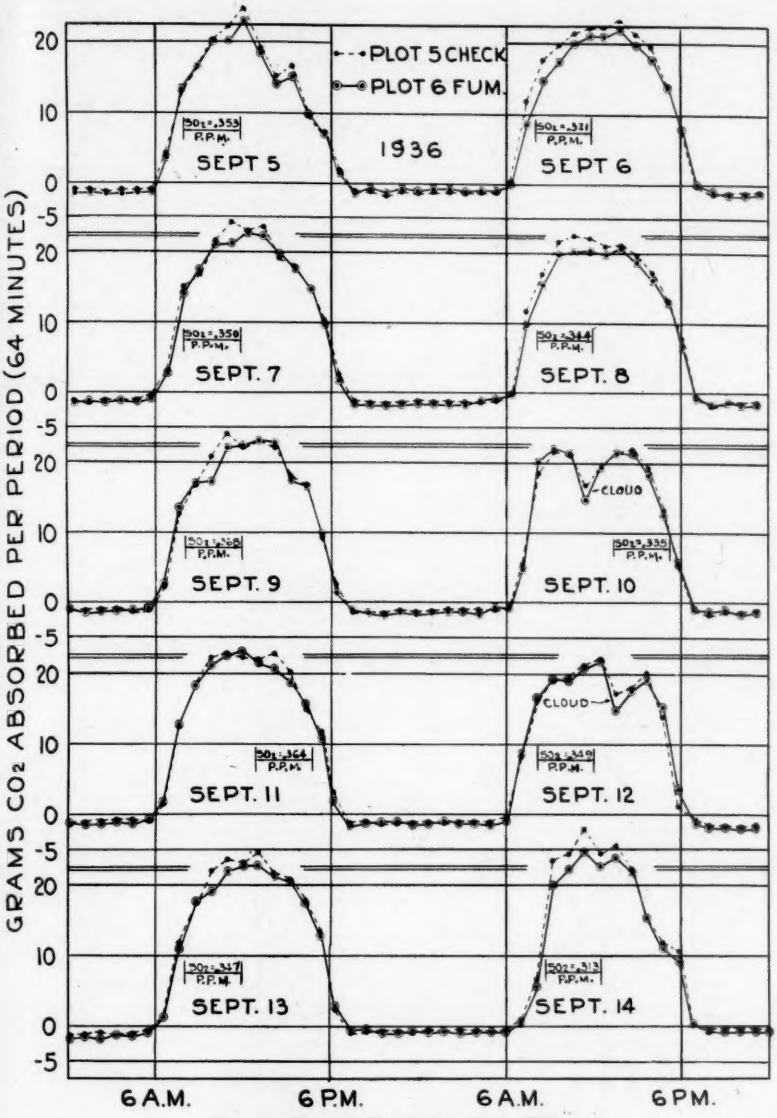

FIG. 6. Chart B, September 5–14, 1936.

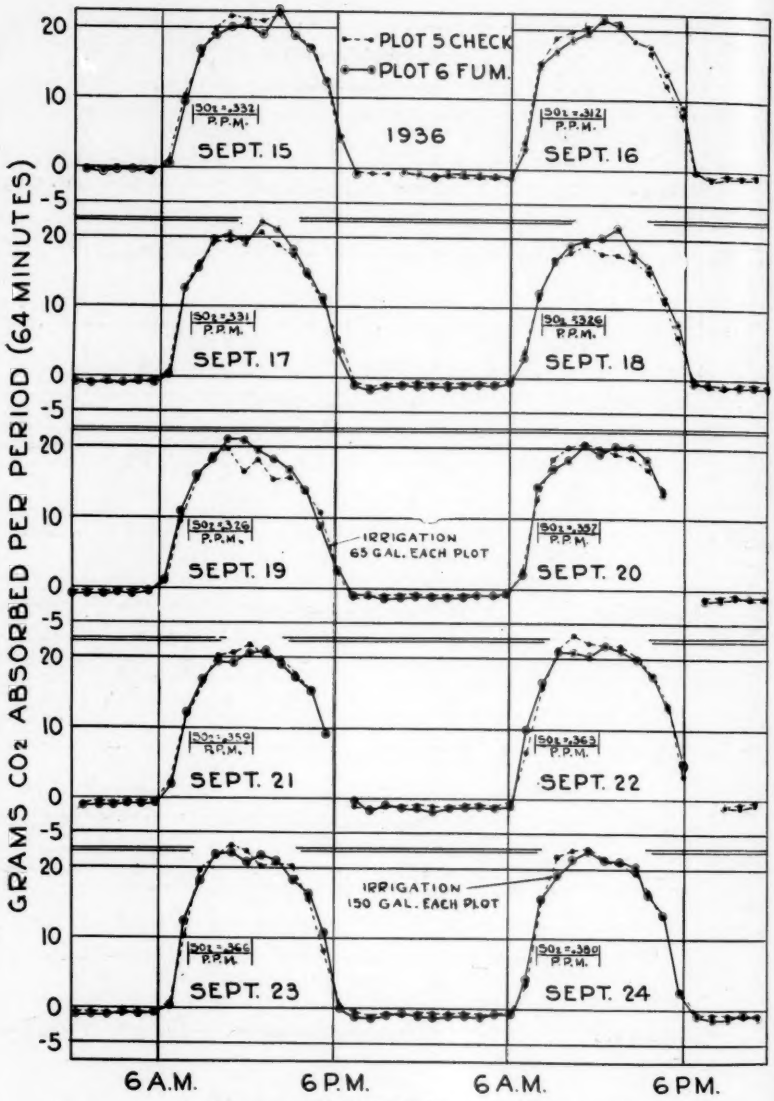

FIG. 6. Chart C, September 15–24, 1936.

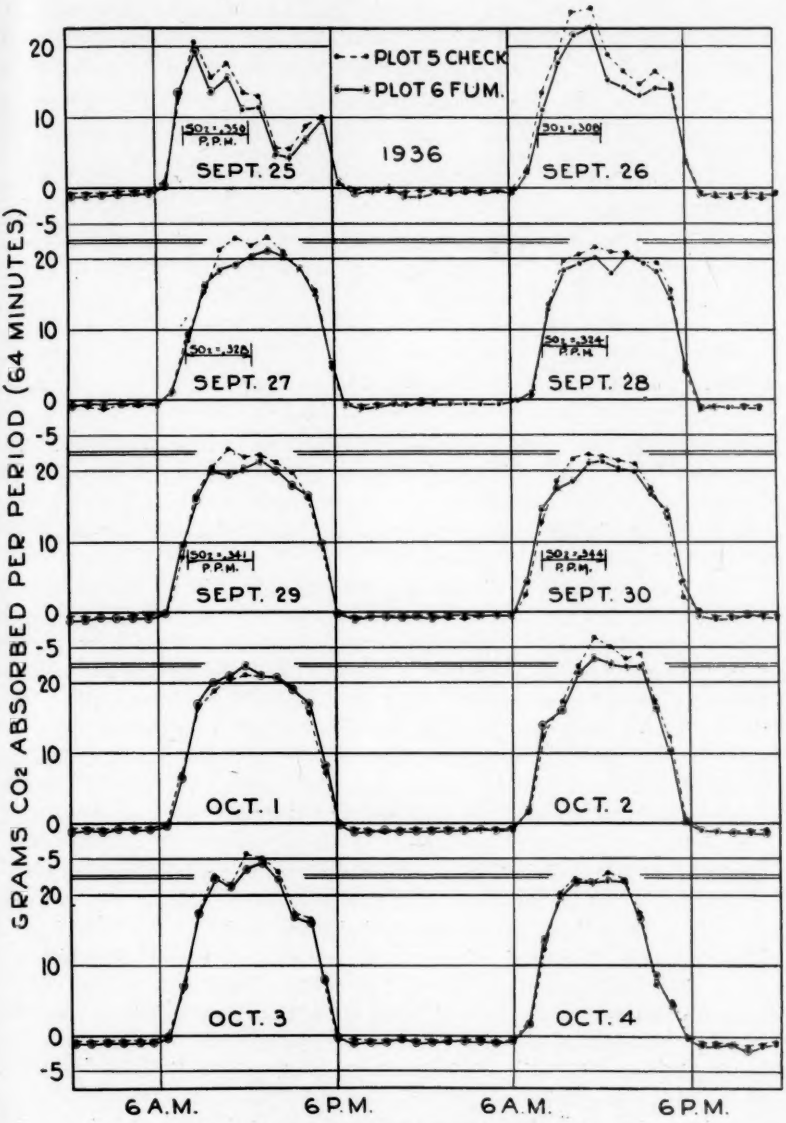

FIG. 6. Chart D, September 25–October 4, 1936.

a result of the drought, and some cold nights (minimum temperature 34° F.), a considerable amount of leaf discoloration and destruction appeared on both plots. Table XIII gives the observed leaf destruction. It will be

TABLE XIII

LEAF AREA ON PLOTS 5 AND 6 SHOWING DISCOLORATIONS FROM VARIOUS CAUSES

DATE (1936)	PLOT 5 (CHECK)			PLOT 6 (FUMIGATED)		
	BLEACHED	CHLOROTIC	TOTAL	BLEACHED	CHLOROTIC	TOTAL
	%	%	%	%	%	%
September 8	.....	5.7	5.7	0.6	5.9	6.5
" 19	.....	3.5	3.5	0.3	2.5	2.8
" 28	2.4	4.8	7.2	0.9	5.5	6.4
October 5	1.0	2.5	3.5	1.7	3.8	5.5

seen that the two plots had nearly the same amounts of lesions. The markings in the fumigation cabinet could not be distinguished from those in the check. Nevertheless, the final measurements, which were the most reliable, show slightly higher values on the treated plot, and the differences could be attributed to the fumigations.

The curves of carbon dioxide exchange are given in figure 6, charts A, B, C, and D, and the data are summarized in table XIV. The curves in figure 6 and table XIV show that from August 30 to September 16, and from September 20 to September 30, apparent assimilation and net assimilation were normal at all times, except the hours of actual fumigation, since the ratios of fumigated to check plots after the fumigations were not significantly different from the ratios before fumigation began. During the time that the fumigation was actually in progress, the rate of assimilation was reduced about 4 per cent. on August 30 and 31, and about 6 per cent. between September 2 and 16. This is from one-third to one-fifth of the amount that the assimilation of both plots was reduced by passing clouds on August 29, 30, 31, September 2, and September 3. During the remainder of each of these days the average assimilation, omitting the hour immediately following the fumigation, was 99 per cent. of the check, which was not a significant reduction. From September 17 until September 19 the treated plot was about 4 per cent. more active than the check in the morning while the sulphur dioxide was present; and during the afternoon when the fumigation was discontinued, it was 7 per cent. more active than the check plot. This was possibly due to the fact that the check plot seemed to be suffering a little more from drought than the treated plot. It is interesting to note that relations between the fumigation and post-fumigation periods were relatively the same on these days as when the plots were adequately watered. On the afternoon of September 19, the plots were irrigated and

on subsequent days the effect of the fumigation again became perceptible, the reduction being about 7 per cent. in the mornings but only 1 per cent. in the afternoons from September 20 to 30. During the final 4-day observation period, after all fumigation was discontinued, the two plots finally came to very close coincidence in their photosynthetic activity, the average value of the treated plot being 98 per cent. of the check during this period The reduction in the amount of net assimilation on 28 days of treatment (not including September 17, 18, and 19) was 3.2 per cent. of the total net assimilation. As 16 per cent. of the latter was utilized for top growth, the estimated effect of these treatments on the yield of the crop was a reduction of about 0.5 per cent. The actual yields were practically identical. The drought certainly did not add to the effect of the fumigation treatment.

The sulphur and chlorophyll analyses on plots 5 and 6 are summarized in table XV. The chlorophyll content of the leaves decreased gradually, plot 6 a little more than plot 5, but not so much as the alfalfa outside the plant chambers. These differences in the chlorophyll content of the leaves are probably without practical significance.

It will be noted that the sulphur content of the leaves of plot 6 increased to about 1.7 per cent. The sulphur content of the upper leaves was always higher in plot 6 than that of the lower leaves, and the older upper leaves contained more sulphur than the younger upper leaves. In the check plot the lower leaves always had more sulphur than the upper leaves. The total excess of sulphur in plot 6 over plot 5, given by the analyses of plant tissue, is 7.44 gm. as compared with 5.91 gm. given by gas analyses in table XII. Again, this excess may be ascribed to an accumulation of this element from earlier fumigations. The amounts of sulphur present in the plant tissue on September 4 have been calculated, using an estimated value for the amount of plant substance present, which is based on comparable plants harvested on August 24 and on subsequent measurements of stem growth. When these sulphur values are subtracted from the total sulphur subsequently found, the excess of this element in plot 6 over plot 5 becomes 5.19 gm., as compared with 5.06 gm. determined by gas analyses. The latter value includes half of the sulphur absorbed on September 4, because the plant samples of September 4 were taken in the middle of the fumigation period. The concordance between these values is very close, but it is likely that plot 6 was being supplied with sulphur from its roots a litttle more rapidly than plot 5, even in this period, because there is always some absorption by the cabinet and the soil.

Effect of very long continuous fumigations with sulphur dioxide of low concentration on carbon dioxide exchange

Three series of long continuous fumigations with different low concentrations of sulphur dioxide—one of them lasting 45 days—were conducted

TABLE XIV

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 5 AND 6—1936

DATE (1936)		TOTAL APPARENT ASSIMILATION CO_2			NIGHT RESPIRATION CO_2			NET ASSIMILATION CO_2		
		PLOT 5 CHECK	PLOT 6 FUMIGATED	RATIO 6/5	PLOT 5 CHECK	PLOT 6 FUMIGATED	RATIO 6/5	PLOT 5 CHECK	PLOT 6 FUMIGATED	RATIO 6/5
		gm.	*gm.*	%	*gm.*	*gm.*	%	*gm.*	*gm.*	%
August	24–29	900.3	910.3	101	82.7	86.7	105	817.6	823.6	101
August	30–31*	.....	.....	102[a]	.....	.....	.....	.....	.....	.....
	"	.....	.....	96[b]	.....	.....	.....	.....	.....	.....
	"	.....	.....	101[c]	.....	.....	.....	.....	.....	.....
	"	303.3	298.9	99[d]	40.8	38.8	95	262.5	260.1	99
September	1	186.3	192.0	103	19.2	15.4	80	167.1	176.6	106
September	2–16*	.....	.....	98[a]	.....	.....	.....	.....	.....	.....
	"	.....	.....	94[b]	.....	.....	.....	.....	.....	.....
	"	.....	.....	99[c]	.....	.....	.....	.....	.....	.....
	"	2755.5	2638.7	96[d]	170.2	186.3	109	2585.3	2452.4	95
September	17–19*	.....	.....	101[a]	.....	.....	.....	.....	.....	.....
	"	.....	.....	104[b]	.....	.....	.....	.....	.....	.....
	"	.....	.....	107[c]	.....	.....	.....	.....	.....	.....
	"	490.9	516.4	105[d]	23.1	29.1	126	467.8	487.3	104
September	20–30*	.....	.....	104[a]	.....	.....	.....	.....	.....	.....
	"	.....	.....	93[b]	.....	.....	.....	.....	.....	.....
	"	.....	.....	99[c]	.....	.....	.....	.....	.....	.....
	"	1888.3	1816.8	96[d]	93.6	108.7	116	1794.7	1708.1	95
October	1– 4	688.5	676.2	98	37.7	46.2	123	650.8	630.0	97
Totals	.....	7213	7049	98	467	511	109	6746	6538	97

Equivalent dry matter (44 per cent. carbon) (grams)	4180	4052	.....
Dry matter October 5 at harvest (grams)	1480	1508	102%
Dry matter August 24 (estimated from weight of equivalent plot harvested August 24) (grams)	845	845	.....
Top growth during experiment (grams)	635	663	.....
Top growth—percentage of total CO_2 assimilated	15.2	16.4	.....
Probable root increment (grams)	3545	3389	.....

* A 4.25-hour fumigation on plot 6 each morning on these dates, except September 2, 3, 10, and 11, when fumigation was given in the afternoon. [a] Before fumigation. [b] During fumigation. [c] After fumigation. [d] Total for the period.

TABLE XV

afternoon. [a] Before fumigation. [b] During fumigation. [c] After fumigation. [d] Total for the period.

TABLE XV

ANALYSES OF PLOTS 5 AND 6 FOR SULPHUR AND CHLOROPHYLL (DRY BASIS)—1936

DATE (1936)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 5 (CHECK)			PLOT 6 (FUMIGATED)			PLOT 5 CHECK	PLOT 6 FUMIGATED	OUTSIDE* CHECK
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)			
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
September 4	Leaves	480[a]	0.572	2.75	480[a]	0.990	4.75	1.78	1.70	1.61
" 4	Stems	550[a]	0.199	1.09	555[a]	0.242	1.34	.....	.....	.....
" 4	Total	1030	0.373	3.84	1035	0.588	6.09	.....	.....	.....
" 8	Leaves	18.0	0.566	0.102	21.2	1.168	0.248	1.83	1.74	1.36
" 14	Upper leaves	22.2	0.485	0.108	21.5	1.400	0.301	1.75	1.67	1.37
" 14	Lower "	7.3	0.775	0.057	6.5	0.860	0.056	1.72	1.66	.....
" 19	Upper leaves	20.6	0.463	0.095	21.0	1.647	0.346	1.56	1.27	1.29
" 19	Lower "	7.4	0.630	0.047	9.9	1.418	0.140	1.61	1.48	1.60
" 28	Upper leaves	22.8	0.437	0.100	24.9	1.758	0.438	1.51	1.28	1.05
" 28	Lower "	6.0	0.595	0.036	8.6	1.510	0.130	1.67	1.39	1.22
October 5	Upper secondary	235	0.450	1.058	232	1.670	3.875	1.42	1.36	1.29
" 5	Upper primary	129	0.576	0.744	152	1.748	2.655	1.58	1.34	.....
" 5	Lower leaves	92	0.628	0.578	65	1.391	0.905	1.47	1.20	1.34
September 4	Stems	.....	.....	.....	.....	.....	.....	0.28	0.31	0.31
" 8	"	21.1	0.206	0.043	28.0	0.269	0.075	0.27	0.27	0.28
" 14	"	43.3	0.156	0.068	42.4	0.238	0.101	0.30	0.32	.....
" 19	"	41.8	0.168	0.070	41.0	0.238	0.098	0.26	0.31	.....
" 28	"	43.8	0.166	0.073	47.8	0.265	0.127	0.26	0.26	.....
October 5	"	640	0.130	0.831	652	0.250	1.630	0.34	0.38	0.31
" 5	Dead tissue	72	0.405	0.292	87	0.710	0.618	.....	.....	.....
" 5	Leaves and stems	.....	0.293	.....	.....	0.824	.....	.....	.....	.....
	Total sulphur September 8 to October 5		4.30				11.74			
	Less: sulphur of September 4		3.84				6.09			
	Increment of sulphur September 8 to October 5		0.46				5.65			
	Excess of sulphur in plot 6 over plot 5						7.44			
	Excess of sulphur in plot 6 over plot 5 after September 4						5.19			

* Comparable alfalfa grown adjacent to and outside of plant chambers. [a] Estimated.

TABLE XVI

FUMIGATION DATA OF PLOT 8—1935

DATES	DURATION	AVERAGE AIR VELOCITY EACH DAY			AVERAGE SO_2 CONCENTRATION EACH DAY				WEIGHT OF SO_2 ABSORBED EACH DAY	
					INTAKE		OUTLET			
			DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
			l.p.m.	*l.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*gm.*	*gm.*
September 18	528 Hours	Minimum*	3260	545	0.217	0.149	0.072	0.062	0.462	0.101
to	or	Maximum*	3630	945	0.254	0.276	0.135	0.171	0.683	0.224
October 10	22 Days	Average	3347	766	0.232	0.240	0.114	0.112	0.581	0.173
Average percentage of absorption									51%	53%
Average rate of absorption (mg. SO_2 per minute per 1000 gm. of leaves)									1.4	0.38
Total SO_2 absorbed (grams)									16.6	
Total S absorbed (grams)									8.3	

* Maximum and minimum values in this table were taken from many different fumigations.

at Logan, Utah, in 1935, for the purpose of determining the maximum effects such concentrations could have under the most drastic conditions of application.

G. EFFECT OF CONTINUOUS FUMIGATION AT 0.24 P.P.M.—Plots 7 and 8 were under observation from September 12 to October 15, 1935. The variety of alfalfa in these plots was Utah Common. The fumigation data for plot 8 are given in table XVI. The plot was fumigated continuously from September 18 to October 10—528 hours, or about 22 days. The average intake concentration was 0.236 p.p.m., the average outlet was 0.113 p.p.m., and the mean was 0.175 p.p.m.

The total daily values of apparent assimilation and respiration for plots 7 and 8 are presented in figure 7, and the data are summarized in table XVII. Detailed carbon dioxide exchange curves are given in figure 8, charts A, B, and C. It will be noted that the treated plot was somewhat more vigorous than the check plot at the beginning of the experiment. Accordingly, the ratios of the carbon dioxide values for the two plots have been adjusted to 100 per cent. for the period immediately preceding the fumigation treatment in order to facilitate the comparison of the two plots. During the first 3 days of the fumigation treatment, the rate of apparent assimilation of the treated plot was 1 per cent. less than the comparable rate before the beginning of the treatment. Then the rate fell to 97 per cent. of the check for the next 3 days, to 88 per cent. for 3 days, to 85 per cent. for 7 days, and during the last 6 days of the fumigation to 76 per cent. Finally, the activity rose to a level of about 81 per cent. of the check in the period following the fumigation.

On the treated plot a few bleached markings were first noted 8 days after the fumigation commenced. It was also noted that the older primary leaves were becoming chlorotic. During the next 10 days no new acute markings were found, but many of the older leaves became more chlorotic, until they finally dried up and dropped off. The check plot was nearly free of this type of lesion. During the last 4 days of the fumigation, more acute markings were produced, being generally distributed over the plot. At harvest, plot 8 had 1.7 per cent. of acute markings and 7 per cent. of chlorotic markings. The older primary leaves had 12 per cent. of chlorotic markings. The leaves of the check plot had 6 per cent. of chlorotic discoloration, but no dead lesions. Weather conditions were doubtless responsible for the discolorations on the check plot and for about an equal amount of lesions on the treated plot, leaving only the acute markings and some of the chlorotic markings, particularly on the older leaves, to be ascribed to the fumigation. It will be observed in table XVII that at harvest time, October 16, the weight of functioning leaves on the check plot was 502 gm. as compared with 428 gm. on the treated plot. This reduction in the weight

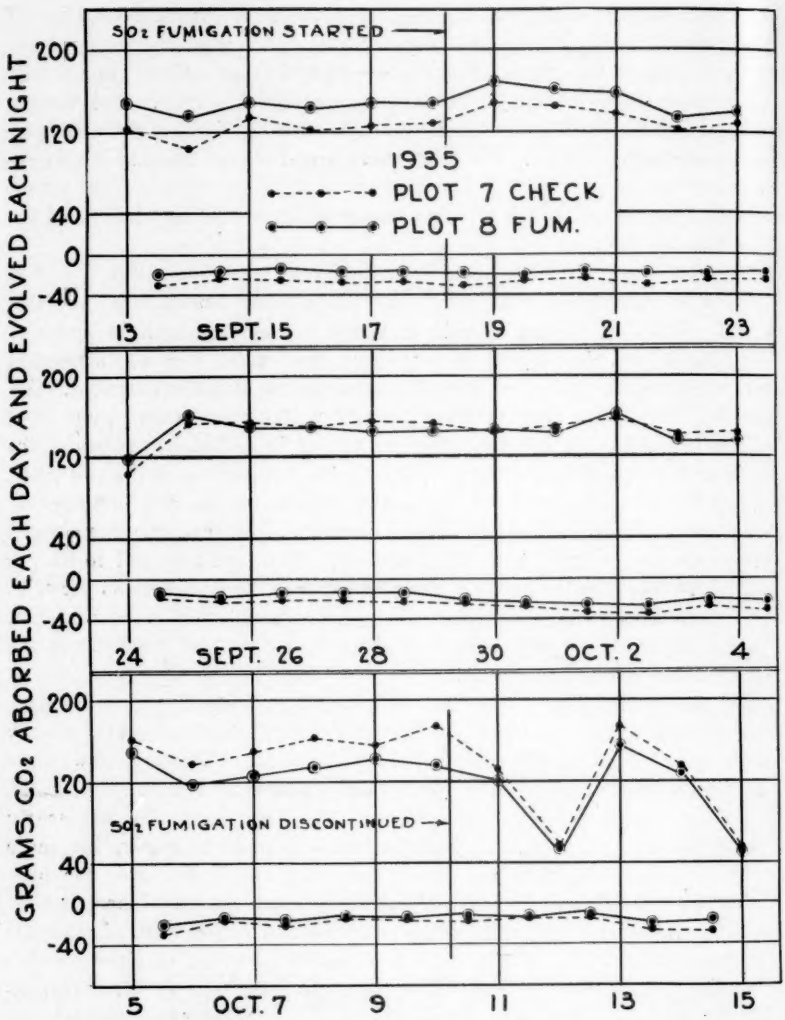

FIG. 7. Total daily apparent assimilation and respiration of plots 7 and 8 at Logan, Utah (1935). Plot 8 was fumigated continuously from September 18 to October 10 (22 days) with 0.235 p.p.m. SO_2.

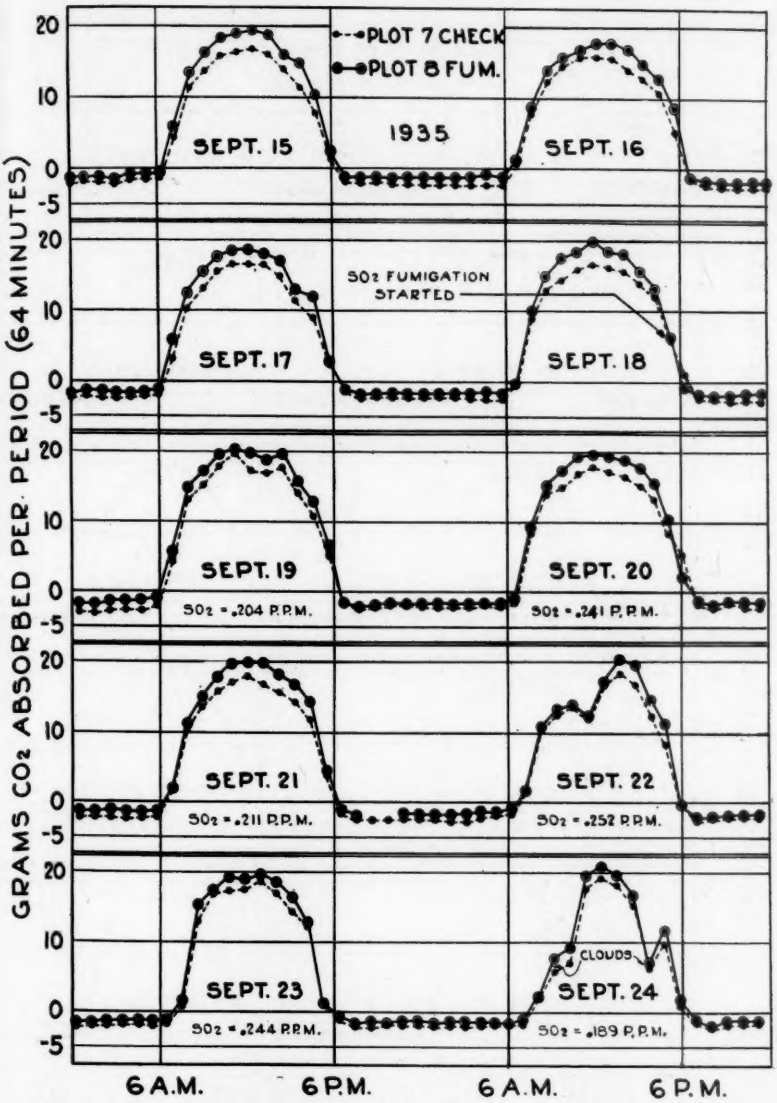

FIG. 8. Chart A, September 15–24, 1935. Apparent assimilation and respiration of plots 7 and 8 at Logan, Utah. Plot 8 was fumigated continuously from September 18 to October 10 (22 days) with 0.235 p.p.m. SO_2.

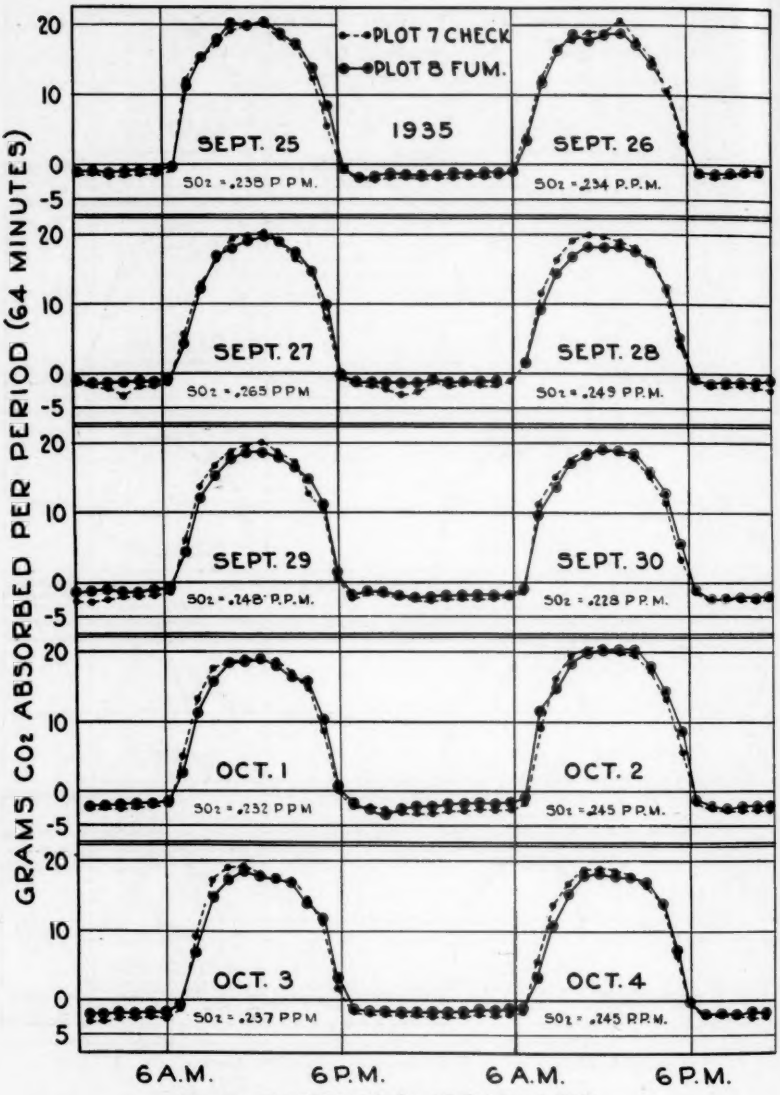

FIG. 8. Chart B, September 25–October 4, 1935.

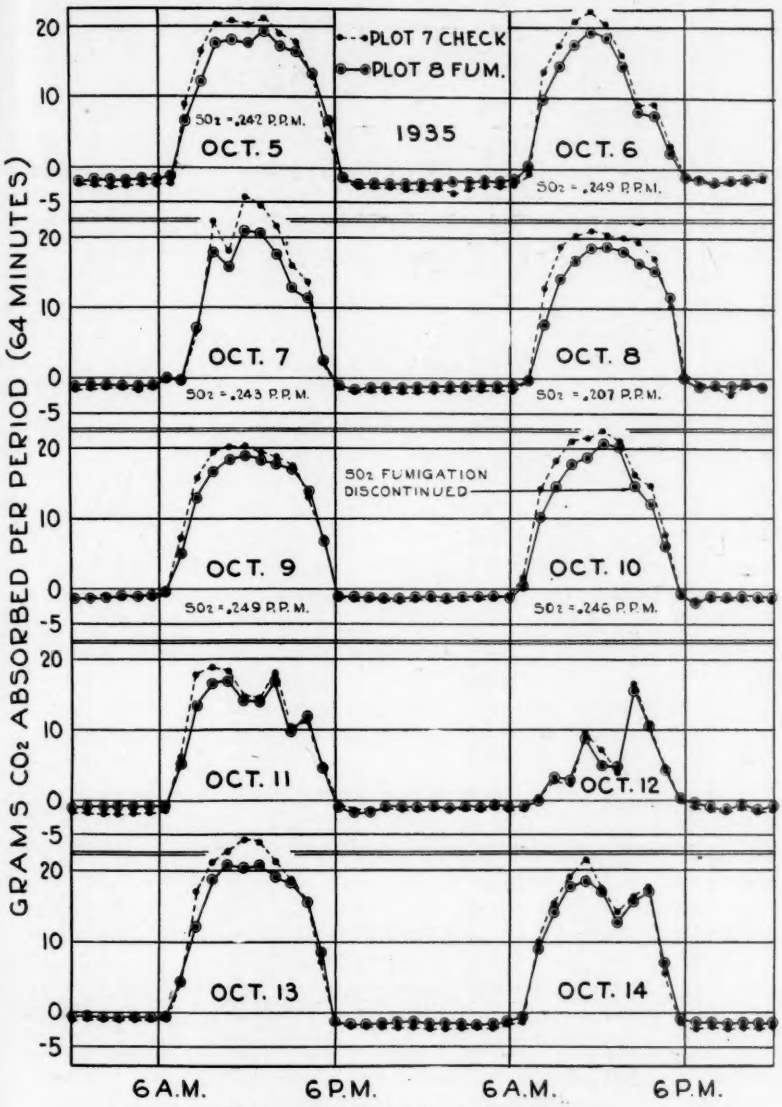

FIG. 8. Chart C, October 5–14, 1935.

TABLE XVII

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA
PLOTS 7 AND 8—1935

DATES (1935)	TOTAL APPARENT ASSIMILATION CO_2				NIGHT RESPIRATION CO_2				NET ASSIMILATION CO_2			
	PLOT 7 CHECK	PLOT 8 FUMIGATED	RATIO 8/7 ACTUAL	RATIO 8/7 ADJUSTED	PLOT 7 CHECK	PLOT 8 FUMIGATED	RATIO 8/7 ACTUAL	RATIO 8/7 ADJUSTED	PLOT 7 CHECK	PLOT 8 FUMIGATED	RATIO 8/7 ACTUAL	RATIO 8/7 ADJUSTED
	gm.	*gm.*	%	%	*gm.*	*gm.*	%	%	*gm.*	*gm.*	%	%
Before fumigation												
9/13– 9/15	365	438	120	105	74	47	64	100	291	391	134	105
9/16– 9/18	380	442	116	102	54	35	65	101	326	407	125	98
9/18	.....	.....	114	100	.....	.....	64	100	.....	.....	128	100
Continuous fumigation started at 5:06 P.M. Sept. 18—average SO_2 concentration 0.236 p.p.m.												
9/19– 9/21	434	490	113	99	107	71	66	103	327	419	128	100
9/22– 9/24	356	394	111	97	69	50	72	113	287	344	120	94
9/25– 9/27	460	461	100	88	61	44	72	113	399	417	105	82
9/28–10/ 4	1058	1028	97	85	194	144	74	116	864	884	102	80
10/ 5–10/10	925	804	87	76	118	91	77	120	807	713	88	69
Average	.....	.....	98	86	.....	.....	73	114	.....	.....	103	81
Fumigation discontinued at 2:48 P.M. Oct. 10												
10/11–10/14	504	464	92	81	79	56	71	111	425	408	96	75
Totals	4482	4521	101	89	756	538	71	111	3726	3983	107	84

Equivalent dry matter (grams) (44 per cent. carbon)			2312	2470
Functioning tissue (grams)	Plot 7	Plot 8		
Samples for analysis October 3	40[a]	32[a]		
	50[b]	49[b]		
Harvest October 16	502[a]	428[a]		
	682[b]	654[b]		
Dead tissue October 16 (grams)	172	320		
Total tissue to October 16 (grams)			1446	1483
Dry matter September 12 (estimated, grams)			644	715
Top growth during experiment (grams)			802	768
Top growth—percentage of total CO_2 assimilated			34.7	31.1
Probable root increment (grams)			1510	1702

[a] Leaves. [b] Stems.

of functioning leaves, together with the greater amount of leaf area marked on the leaves that were left, and perhaps also their somewhat lowered chlorophyll content, indicated later, can account for the reduced photosynthetic activity. The estimated reduction of apparent assimilation which can be ascribed to the fumigation treatment is 11 per cent. of the total apparent assimilation, or 16 per cent. of the total net assimilation. The latter is equivalent to a reduction in top growth of about 8 per cent., since in this experiment only one-third of the net assimilation was converted into top growth. Slightly more top growth was actually found on plot 8 than on plot 7, due perhaps to the more vigorous activity noted at the beginning of the experiment.

Figure 7 and table XVII show a rather large and consistent difference in the rate of respiration of the two plots. This difference is also a noticeable feature of the two following 1935 experiments, whereas in the 1933 and 1936 experiments the pairs of plots exhibited consistently close agreement. The reason for the differences in respiration noted in 1935 is not known. As pointed out in the previous paper (8) no account was taken in this work of "soil respiration", though an appreciable and varying amount is known to exist. The plantings in 1935 were only one year old, and there is a possibility that the rate of "soil respiration" was different on the pairs of plots at that time, due perhaps to unequal amounts of undecomposed manure, but subsequently became equal. It may also be noted that in 1935, the remains of the preceding crop had not been cleared off as thoroughly as in 1936, suggesting the possibility of unequal amounts of decomposition of organic matter on the surface of the soil in 1935. Whatever the cause of the differences, they were sufficiently consistent that they do not present serious difficulties in the interpretation of the photosynthetic data.

Detailed sulphur and chlorophyll analyses were made on the leaves and stems on October 3, and at harvest, October 16 (table XVIII). The leaves were separated into three groups according to age. The sulphur content of the check leaves increased with age. On October 3 the intermediate leaves on the treated plot had the most sulphur, probably because the lower primary leaves were somewhat protected from the gas by the upper leaves which absorbed part of the sulphur dioxide and reduced the concentration of the gas which reached the lower leaves. By October 16 many of the lower leaves had been shed and the remaining primary leaves had the most sulphur. Both the primary and secondary leaves carried about 2 per cent. of sulphur, and the chlorophyll was appreciably lower than in the corresponding check leaves. A sulphur content greater than 2.5 per cent. has never been observed. The tertiary leaves on the other hand with 1.3 per cent. of sulphur were apparently normal in chlorophyll content. The

TABLE XVIII

ANALYSES OF PLOTS 7 AND 8 FOR SULPHUR AND CHLOROPHYLL—1935

DATE (1935)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 7 (CHECK)			PLOT 8 (FUMIGATED)					
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	PLOT 7 CHECK	PLOT 8 FUMIGATED	OUTSIDE* CHECK
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
October 3	Tertiary leaves	17.6	0.414	0.073	13.8	1.216	0.168	1.24	1.19	1.01
" 3	Secondary "	12.8	0.505	0.065	12.0	1.766	0.212	1.36	1.04	1.14
" 3	Primary "	9.3	0.591	0.055	6.0	1.600	0.096	1.38	0.91	1.30
October 16	Tertiary leaves	180	0.434	0.781	168	1.315	2.209	1.29	1.21	1.39
" 16	Secondary "	197	0.517	1.018	173	1.936	3.349	1.33	1.11	1.34
" 16	Primary "	125	0.633	0.791	87	2.130	1.853	1.31	0.92	1.38
" 16	Dead "	82	0.583	0.478	160	0.933	1.493	.....	.....	.....
October 3	Stems	49.7	0.173	0.086	48.7	0.250	0.122	.....	.....	.....
" 16	"	682	0.192	1.309	654	0.278	1.818	.....	.....	.....
" 16	Dead stems	90	0.190[a]	0.171	160	0.250[a]	0.400	.....	.....	.....
" 16	Leaves and stems	.....	0.329	.....	.....	0.852	.....	.....	.....	.....
	Total			4.83			11.72			
	Excess of S. in plot 8 over plot 7						6.89			

* Comparable alfalfa grown adjacent to and outside of plant chambers.

[a] Estimated.

TABLE XIX

FUMIGATION DATA OF PLOT 6—1935

DATES (1935)	DURATION		AVERAGE AIR VELOCITY EACH DAY		AVERAGE SO_2 CONCENTRATION EACH DAY				WEIGHT OF SO_2 ABSORBED EACH DAY	
					INTAKE		OUTLET			
			DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
			l.p.m.	*l.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*gm.*	*gm.*
August 26	628 hours	Minimum*	3765	690	0.152	0.143	0.047	0.041	0.505	0.089
to	or	Maximum*	4150	857	0.218	0.211	0.111	0.135	0.922	0.185
September 21	26 days	Average	3960	768	0.188	0.187	0.077	0.090	0.675	0.128
Average percentage of SO_2 absorbed August 26–September 21									59%	52%
Average rate of absorption (mg. SO_2 per minute per 1000 gm. of leaves)									1.9	0.34
September 25	336 hours	Minimum*	3713	623	0.258	0.234	0.107	0.095	0.683	0.155
to	or	Maximum*	3957	828	0.311	0.315	0.185	0.185	1.000	0.234
October 9	14 days	Average	3880	740	0.292	0.290	0.147	0.144	0.829	0.198
Average percentage of SO_2 absorbed September 25–October 9									50%	50%
Average rate of absorption (mg. SO_2 per minute per 1000 gm. of leaves)									2.2	0.43

SO_2 absorbed August 26–September 21 (grams)	21.12
SO_2 absorbed September 25–October 9 (grams)	14.37
Total SO_2 absorbed (grams)	35.49
Total S absorbed (grams)	17.74

* The maximum and minimum values in the horizontal lines of this table occurred in different fumigations and are, therefore, unrelated.

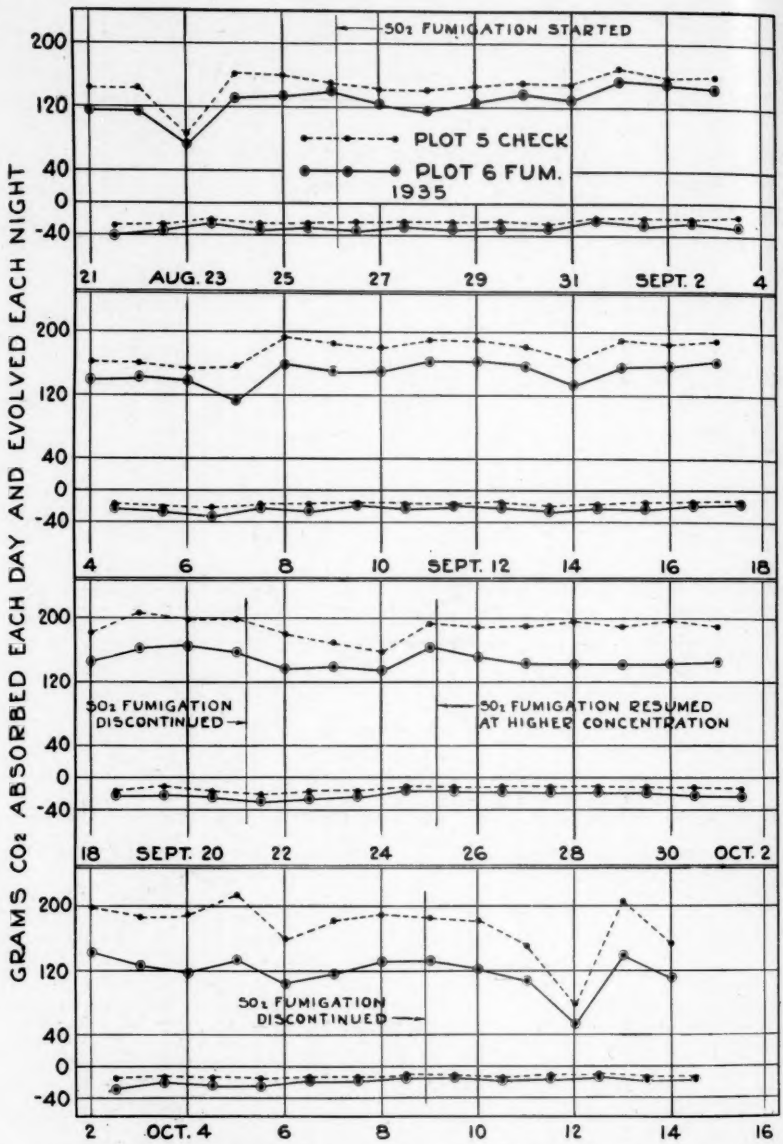
SO₂ FUMIGATION STARTED
PLOT 5 CHECK
PLOT 6 FUM.
1935
GRAMS CO₂ ABSORBED EACH DAY AND EVOLVED EACH NIGHT
200
120
40
0
-40
21 AUG. 23 25 27 29 31 SEPT. 2 4
4 6 8 10 SEPT. 12 14 16 18
SO₂ FUMIGATION DISCONTINUED
SO₂ FUMIGATION RESUMED AT HIGHER CONCENTRATION
18 SEPT. 20 22 24 26 28 30 OCT. 2
SO₂ FUMIGATION DISCONTINUED
2 OCT. 4 6 8 10 12 14 16

FIG. 9. Total daily apparent assimilation and respiration of plots 5 and 6 at Logan, Utah (1935). Plot 6 was fumigated continuously from August 26 to September 21 (26 days) with 0.19 p.p.m. SO_2 and continuously from September 25 to October 9 with 0.29 p.p.m. SO_2.

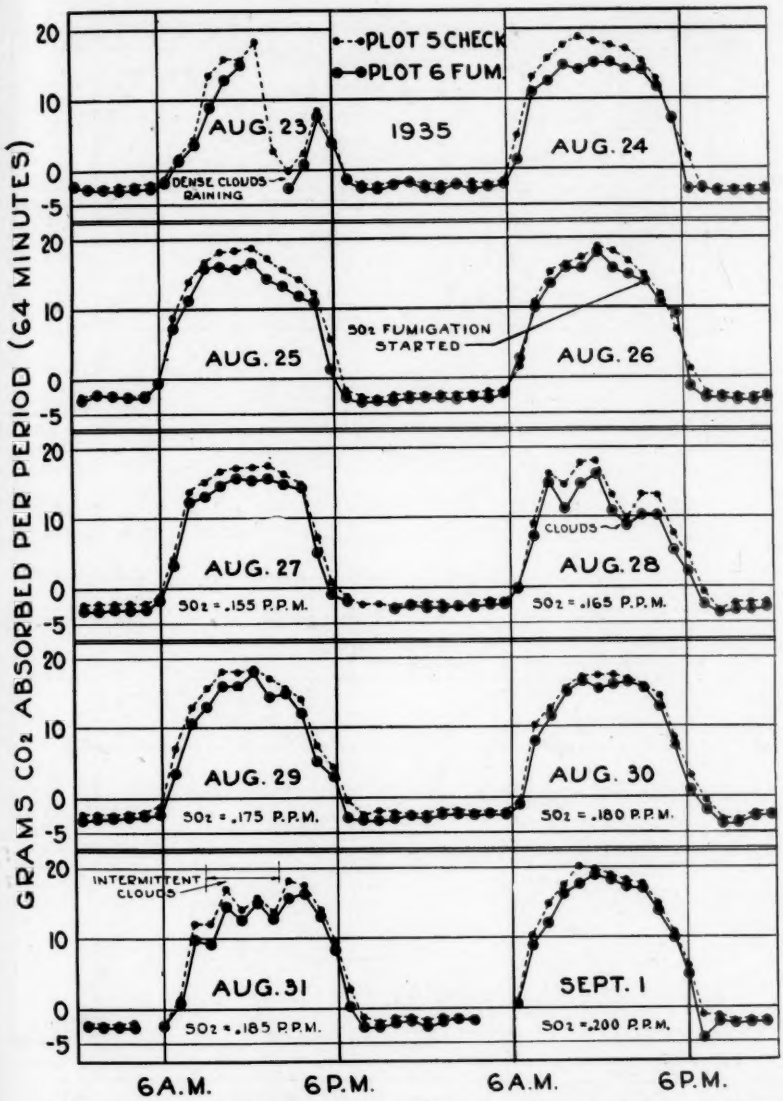

FIG. 10. Chart A, August 23–September 1, 1935. Apparent assimilation and respiration of plots 5 and 6 at Logan, Utah. Plot 6 was fumigated continuously from August 26 to September 21 (26 days) with 0.19 p.p.m. SO_2 and continuously from September 25 to October 9 with 0.29 p.p.m. SO_2.

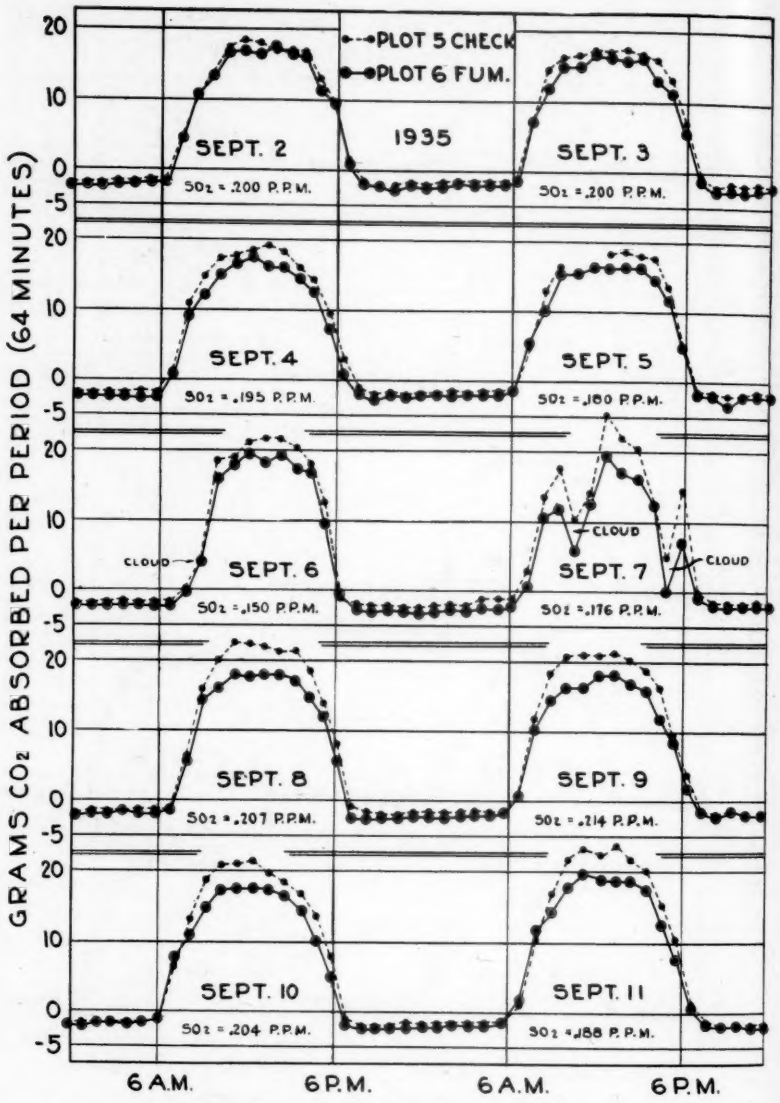

FIG. 10. Chart B, September 2–11, 1935.

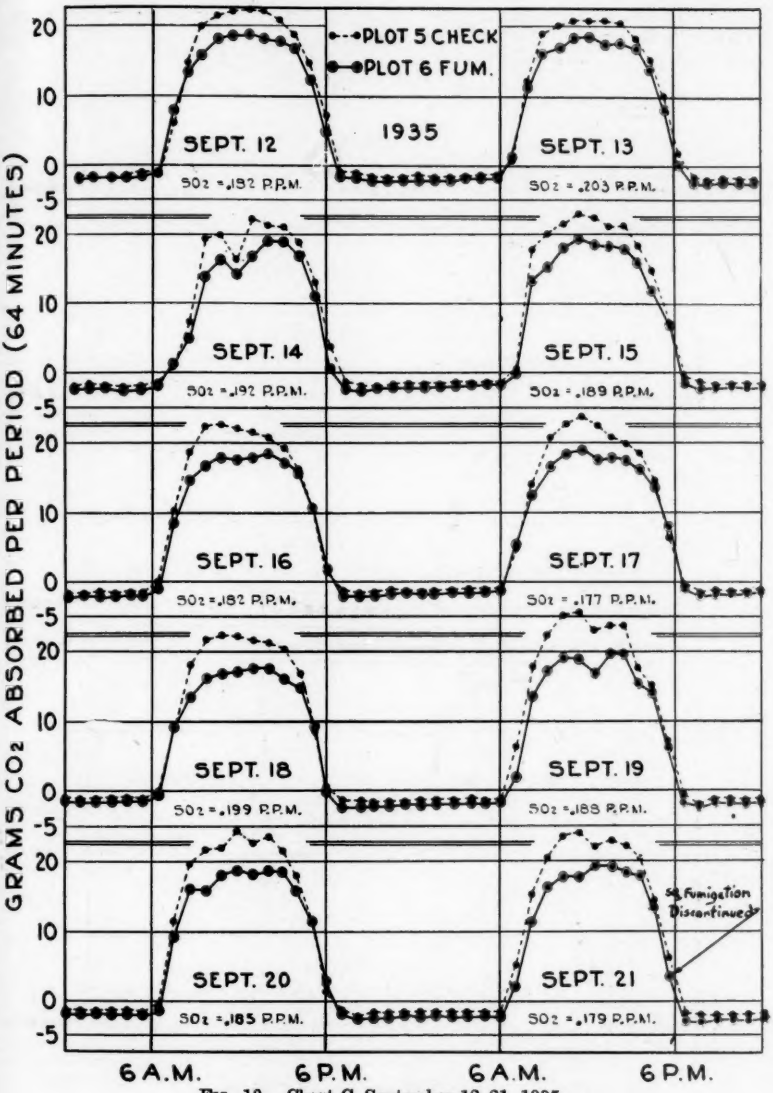

FIG. 10. Chart C, September 12–21, 1935.

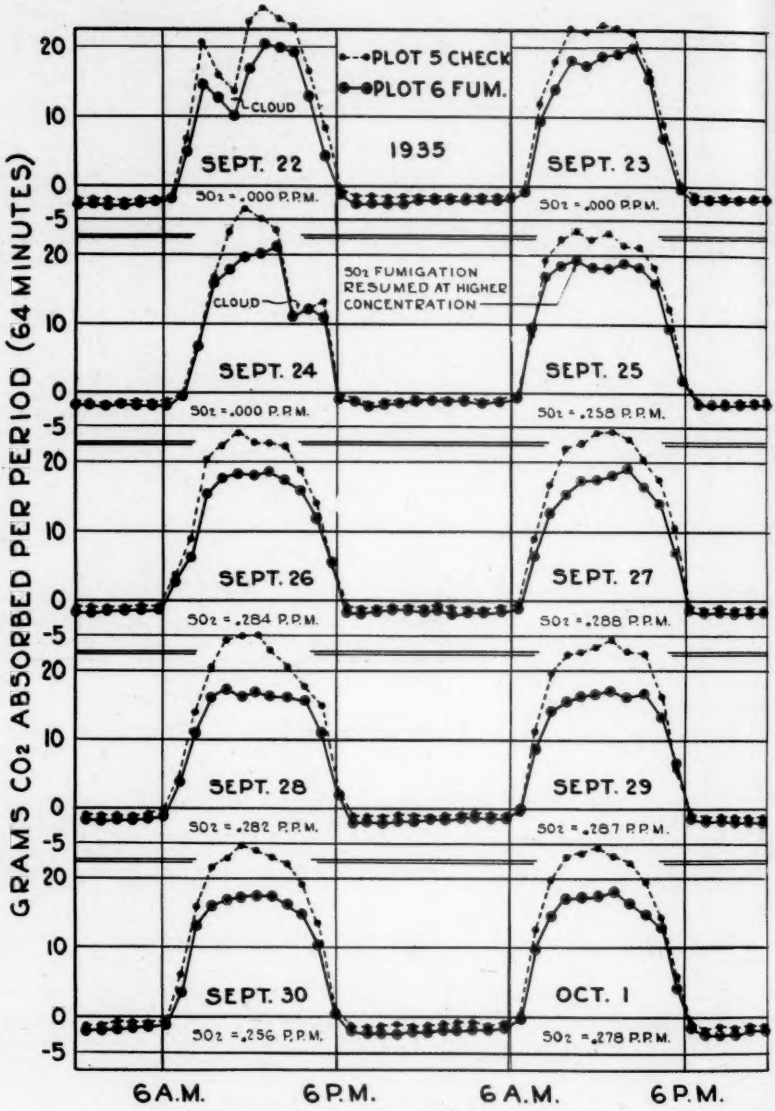

FIG. 10. Chart D, September 22–October 1, 1935.

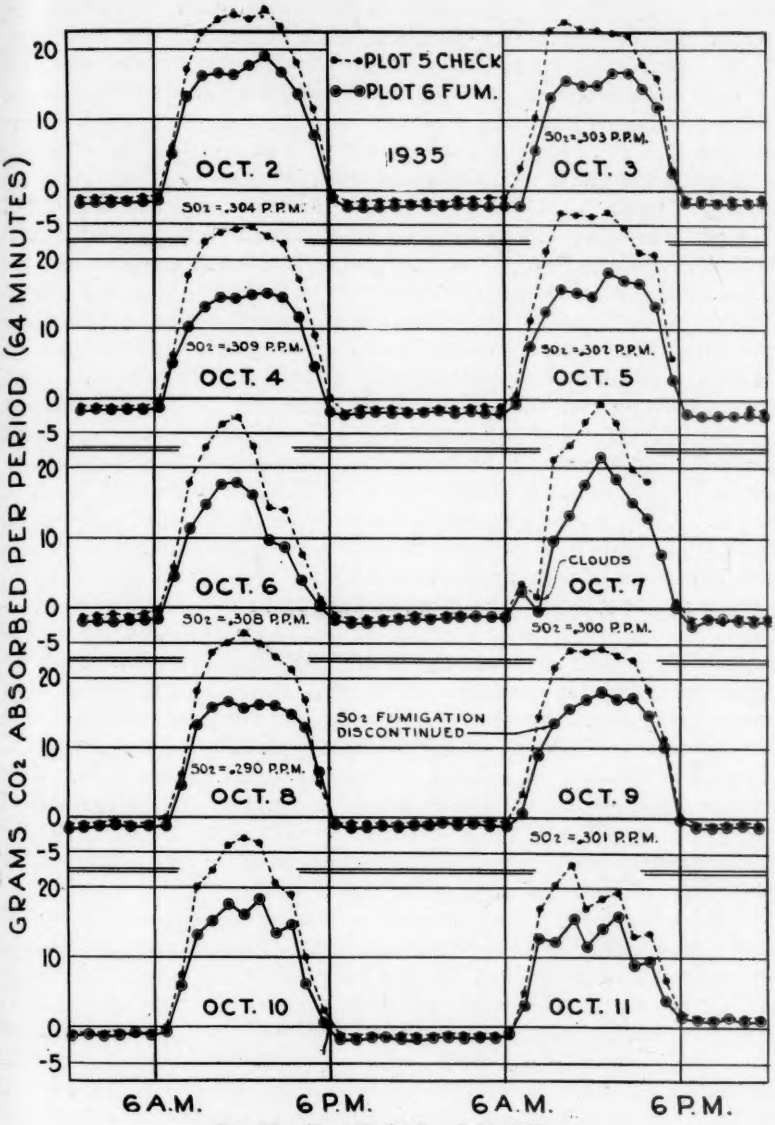

FIG. 10. Chart E, October 2–11, 1935.

excess of sulphur in the plant tissue of plot 8 over plot 7 was 6.90 gm., as compared with 8.3 gm. absorbed as determined by gas analyses.

H. EFFECT OF A CONTINUOUS FUMIGATION OF ABOUT 0.2 P.P.M. FOR ABOUT 26 DAYS FOLLOWED BY A CONTINUOUS FUMIGATION OF ABOUT 0.3 P.P.M. FOR ABOUT 14 DAYS.—Plots 5 and 6 were under observation from August 21 to October 15, 1935. These are the same 2 plots as are described earlier in connection with the fourth 1936 experiment, in which they were given the same plot numbers. The fumigation data are summarized in table XIX. The plot was fumigated continuously from August 26 to September 21—628 hours or about 26 days—with an average intake concentration of 0.188 p.p.m. of SO_2, an average outlet concentration of 0.083 p.p.m., and a mean concentration of 0.136 p.p.m. Then following a rest period of 4 days, the fumigation was resumed from September 25 to October 9—336 hours or about 14 days—with an average intake concentration of 0.291 p.p.m., an average outlet of 0.145 p.p.m., and a mean of 0.218 p.p.m.

The total daily apparent assimilation and respiration of the 2 plots are presented in figure 9, and the data are summarized in table XX. Detailed curves of carbon dioxide exchange are given in figure 10 charts A, B, C, D, and E. As the treated plot exhibited a lower rate of apparent assimilation, and a higher rate of respiration, before the fumigation treatment was applied, the values of the ratios have again been adjusted to facilitate the comparison of the 2 plots. It will be noted that the fumigation caused no reduction in the rate of photosynthesis during the first 11 days. There was actually an increase of about 1 per cent. in the net assimilation. Then the apparent assimilation level fell to 89 per cent. of the check for 3 days, then rose to 95 per cent. for the next 8 days, and finally fell to 90 per cent. during the last 4 days of the first fumigation treatment. During the rest period the level of apparent assimilation rose to 95 per cent. of the check. With the administration of the higher concentration, the level of the treated plot gradually fell until finally it was 73 per cent. of the check. After the fumigation was finally discontinued the photosynthetic level of the treated plot rose to about 79 per cent. of the check.

During the first fumigation period only a few markings were observed on the treated plot, and these were largely confined to the older leaves, which seemed to become yellow and drop off somewhat earlier than on the check plot. After the fumigation was resumed at the higher concentration, however, acute and chlorotic markings were conspicuous on the treated plot and they became progressively more numerous as the fumigation progressed. At harvest all of the leaves were separated from the stems and the leaves were collected into 3 groups, according to age, as primary, secondary, and tertiary. The oldest leaves on plot 6 had 2.4 per cent. acute markings and 12.4 per cent. of chlorotic markings. The average values for the plot were

TABLE XX

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 5 AND 6—1935

TABLE XX

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 5 AND 6—1935

DATE (1935)	TOTAL APPARENT ASSIMILATION CO_2				NIGHT RESPIRATION CO_2				NET ASSIMILATION CO_2			
	PLOT 5 CHECK	PLOT 6 FUMIGATED	RATIO 6/5		PLOT 5 CHECK	PLOT 6 FUMIGATED	RATIO 6/5		PLOT 5 CHECK	PLOT 6 FUMIGATED	RATIO 6/5	
			ACTUAL	ADJUSTED			ACTUAL	ADJUSTED			ACTUAL	ADJUSTED
	gm.	*gm.*	%	%	*gm.*	*gm.*	%	%	*gm.*	*gm.*	%	%
					Before fumigation							
8/21– 8/24	534	432	81	91	104	131	126	96	430	301	70	87
8/25– 8/26	309	274	89	100	49	64	131	100	260	210	81	100
	Continuous fumigation started at 2: 20 P.M. August 26—average SO_2 concentration 0.188 p.p.m.											
8/27– 9/ 6	1700	1507	89	100	221	296	134	102	1479	1211	82	101
9/ 7– 9/ 9	533	421	79	89	51	66	129	98	482	355	74	91
9/10– 9/17	1469	1239	84	95	129	173	134	102	1340	1066	80	98
9/18– 9/21	786	630	80	90	69	99	143	109	717	531	74	91
Average	.....	.....	85	96	.....	.....	135	103	.....	.....	78	96
	Fumigation discontinued at 6: 40 P.M., September 21											
9/22	179	136	76	85	17	26	153	117	162	110	68	84
9/23– 9/25	521	438	84	95	40	55	137	105	481	383	80	99
	Continuous fumigation resumed at 11: 08 A.M. September 25—average SO_2 concentration 0.291 p.p.m.											
9/26–10/ 2	1344	1009	75	84	87	147	169	129	1257	862	69	85
10/ 3–10/ 8	1115	727	65	73	82	121	148	113	1033	606	59	73
Average	.....	.....	71	80	.....	.....	159	121	.....	.....	64	79
	Fumigation discontinued at 11 A.M., October 9											
10/ 9–10/14	957	666	70	79	75	96	128	98	882	570	65	80
Totals	9447	7479	79	89	924	1274	138	105	8523	6205	73	90

	Plot 5	Plot 6		
Equivalent dry matter (grams) (44% carbon)			5284	3847
Functioning tissue (grams)				
Interim samples for analysis	98[a]	91[a]		
	132[b]	130[b]		
Harvest October 15	597[a]	511[a]		
	766[b]	724[b]		
Dead tissue October 15 (grams)	280	340		
Total tissue October 15 (grams)			1873	1796
Dry matter August 20 (estimated, grams)			600	600
Top growth during experiment (grams)			1274	1196
Top growth—percentage of total CO_2 assimilated			24.1	31.1
Probable root increment (grams)			4010	2651

[a] Leaves. [b] Stems.

1.2 per cent. of acute, and 5.5 per cent. of chlorotic markings. The latter were partly weather discolorations, because plot 5 had about 2 per cent. of the same type, but no bleached discolorations. The amount of marked leaf area remaining on the plants at harvest time is insufficient to account for the observed reduction of about 21 per cent. in the final rate of assimilation. Table XX indicates that there were only 511 gm. of functioning leaves on the treated plot at harvest, as compared with 597 gm. on the check plot. As the weights of stems on the two plots were more nearly equal, the difference between the weights of the leaves probably represents largely leaves that were shed. The reduced amount of functioning leaves with their discolorations and lowered chlorophyll content (table XXI) accounts for the reduced rate of assimilation.

Table XX indicates that during the first fumigation, the rate of net assimilation was reduced an average of 4 per cent., and during the second fumigation 21 per cent. The total reduction for the whole experiment was 10 per cent.

Sulphur and chlorophyll analyses are presented in table XXI. The values and relations of these constituents are quite similar to those of the preceding experiment. A smaller portion (9.79 out of 17.74 gm., tables XXI and XIX) of the total sulphur absorbed in plot 6 was accounted for by these analyses than was accounted for in plot 8. This is probably due to the fact that the fumigation of plot 6 was more protracted than that of plot 8. Some of the absorbed sulphur evidently went into the roots, because in the 1936 experiments, more sulphur was accounted for than was added later. It seems worth while to tabulate the sulphur balance on this crop and the three 1936 crops. This has been done in table XXII, which also includes the analyses of the roots which were dug up in a dormant condition in February, 1937. The air analyses show that in three fumigation experiments, in 1935 and 1936, 24.62 gm. of sulphur were added to plot 6 as sulphur dioxide. The analyses of the plant tissue from the fumigated crops in 1935 and 1936, plus that of the first crop of 1936 which was not fumigated, indicate that plot 6 had 21.59 gm. of sulphur more than plot 5. Each crop on plot 6 in 1936 returned some of the sulphur that was unaccounted for in 1935. There had evidently been a translocation in 1935 of a considerable portion of the added sulphur to the roots. There was still an excess of 0.36 gm. in the roots of plot 6 at the end of the 1936 season. The sulphur finally unaccounted for represents 12 per cent. of the total amount of sulphur dioxide added. This is about the same as the percentage unaccounted for in the other experiments in this paper, which were not complicated by previous fumigations. The soil contained 5.3 p.p.m. of sulphur as soluble sulphate in plot 5 and 5.1 p.p.m. in plot 6. No significant difference could be detected between soil of the two plots, even in the surface

TABLE XXI

ANALYSES OF PLOTS 5 AND 6 FOR SULPHUR AND CHLOROPHYLL—1935

DATE (1935)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 5 (CHECK)			PLOT 6 (FUMIGATED)			PLOT 5 CHECK	PLOT 6 FUMIGATED	OUTSIDE* CHECK
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)			
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
September 10	Leaves	14.5	0.395	0.057	13.1	1.120	0.147	.....	.....	.....
" 17	"	35.2	0.389	0.137	32.4	1.390	0.450	1.32	1.23	1.18
" 25	"	38.2	0.396	0.151	32.7	1.330	0.435	1.33	1.17	1.12
October 15	Tertiary leaves	238	0.365	0.869	175	1.628	2.852	1.30	1.16	1.10
" 15	Secondary "	242	0.421	1.019	261	1.972	5.147	1.37	1.00	1.04
" 15	Primary "	117	0.511	0.598	75	2.265	1.699	1.26	1.01	1.03
" 15	Dead leaves	80	0.578	0.462	100	1.276	1.276	.....	.....	.....
September 10	Stems	18.8	0.165	0.031	23.2	0.198	0.046	.....	.....	.....
" 17	"	50.3	0.106	0.053	46.6	0.266	0.124	.....	.....	.....
" 25	"	52.1	0.162	0.084	46.4	0.186	0.086	.....	.....	.....
October 15	"	766	0.141	1.080	724	0.258	1.868	.....	.....	.....
" 15	Dead stems	200	0.140[a]	0.280	240	0.20[a]	0.480	.....	.....	.....
" 15	Leaves and stems	.....	0.262	.....	.....	0.937	.....	.....	.....	.....
	Total			4.821			14.610			
	Excess of S in plot 6 over plot 5						9.79			

* Comparable alfalfa grown adjacent to and outside of plant chambers.
[a] Estimated.

TABLE XXII

BALANCE SHEET OF SULPHUR IN PLOT 6 (FUMIGATED IN 1935 AND 1936) AS COMPARED WITH PLOT 5 (CHECK)

DATE OF HARVEST	PLANT TISSUE	SO_2 ADDED TO PLOT 6 (GAS ANALYSIS) (S)	TOTAL SULPHUR (S) IN TISSUE			EXCESS OF SULPHUR IN PLOT 6 OVER PLOT 5, MINUS SULPHUR ADDED AS SO_2
			PLOT 5	PLOT 6	DIFFERENCE	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
October 15, 1935	Third crop	17.74	4.82	14.61	9.79	−7.95
June 26, 1936	First crop	None	6.22	7.50	1.28	+1.28
August 3, 1936	Second crop	0.97	5.47	8.19	2.72	+1.75
October 15, 1936	Third crop	5.91	4.30	11.74	7.44	+1.53
February 7, 1937	Roots		5.58	5.94	0.36	+0.36
Totals		24.62	26.39	47.98	21.59	−3.03

inch. In preparing the roots for analysis, they were washed with distilled water. Soil, approximately equal in weight to the roots, was thus removed. This adhering soil from each plot contained 74 p.p.m. of sulphur as sulphate, which represents about 14 times as much soluble sulphate in the soil clinging to the roots as in the average soil of the plot. This suggests a passage outward of the sulphate from the roots to soil.

I. EFFECT OF A CONTINUOUS FUMIGATION OF 0.14 P.P.M. FOR ABOUT 45 DAYS.—Plots 3 and 4 were under observation from July 16 to September 5, 1935. These were the same plots that were described above in connection with a 1936 experiment. The fumigation data for plot 4 are summarized in table XXIII. The plot was given a continuous treatment with sulphur dioxide from July 20 to September 3—1078 hours or about 45 days. The average intake concentration was 0.141 p.p.m. and the average outlet concentration 0.050 p.p.m., or a mean of 0.095 p.p.m.

The total daily values of the apparent assimilation and respiration on these plots are presented in figure 11, and are summarized in table XXIV. The detailed daily charts for this experiment are given in figure 12 charts A, B, C, D, and E. The curves of photosynthesis indicate that plot 4 was somewhat less active than plot 3 before the fumigation treatment started, and again the ratios have been adjusted to facilitate comparison. In this case the initial fumigation period was used as reference, because two days in the prefumigation period, July 18 and 19, had almost as high an assimilation rate as obtained after July 21. The machines did not operate smoothly on July 20, and the differences noted on that date are probably too great. During the first 8 days of the fumigation, the rate of apparent assimilation of the treated plot was somewhat greater in relation to the check plot than

TABLE XXIII

FUMIGATION DATA OF PLOT 4—1935

DATES	DURATION	AVERAGE AIR VELOCITY EACH DAY			AVERAGE SO_2 CONCENTRATION EACH DAY				WEIGHT OF SO_2 ABSORBED EACH DAY	
					INTAKE		OUTLET			
			DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
			l.p.m.	*l.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*gm.*	*gm.*
July 20	1078 hours	Minimum*	2830	731	0.09	0.07	0.017	0.015	0.398	0.066
to	or	Maximum*	3782	1330	0.19	0.26	0.073	0.112	0.736	0.264
September 3	45 days	Average	3452	883	0.146	0.136	0.050	0.050	0.565	0.123
Average percentage of SO_2 absorbed									68%	65%
Average rate of absorption (mg. SO_2 per minute per 1000 gm. of leaves)									1.4	0.33
Total SO_2 absorbed (grams)									30.94	
Total S absorbed (grams)									15.47	

* The maximum and minimum values in the horizontal lines of this table occurred in different fumigations and are, therefore, unrelated.

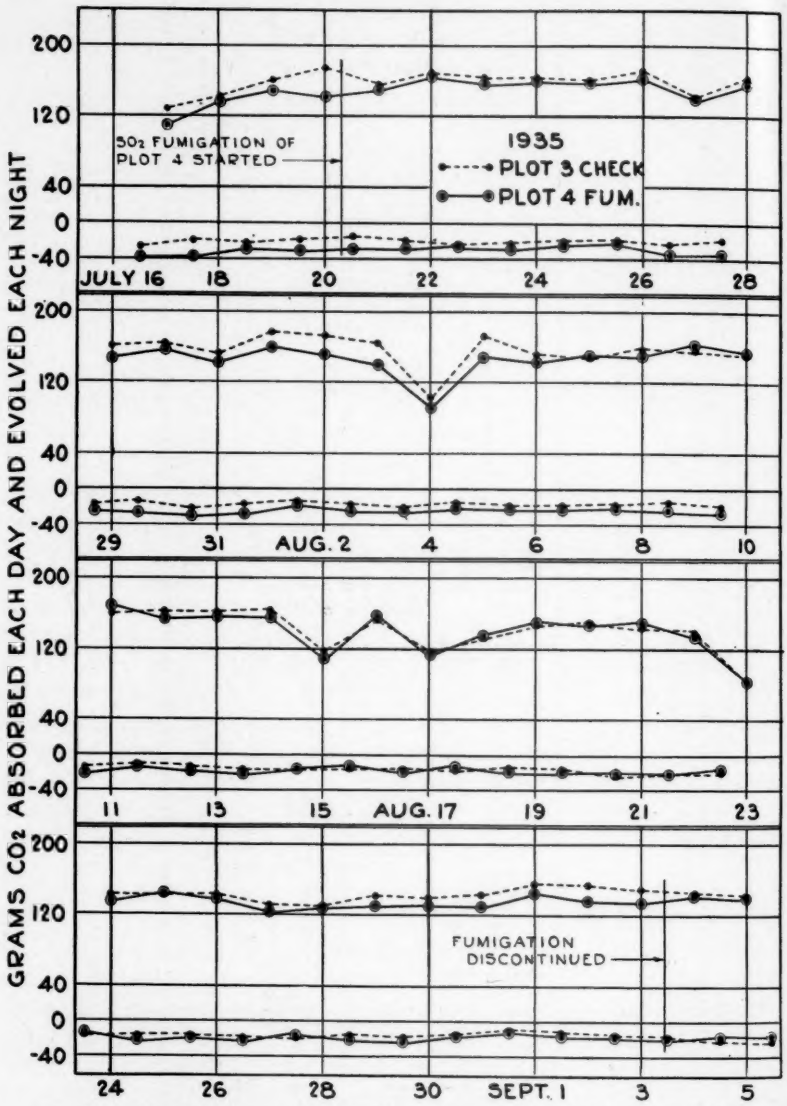

Fig. 11. Total daily apparent assimilation and respiration of plots 3 and 4 at Logan, Utah (1935). Plot 4 was fumigated continuously from July 20 to September 3 (45 days) with 0.14 p.p.m. SO_2.

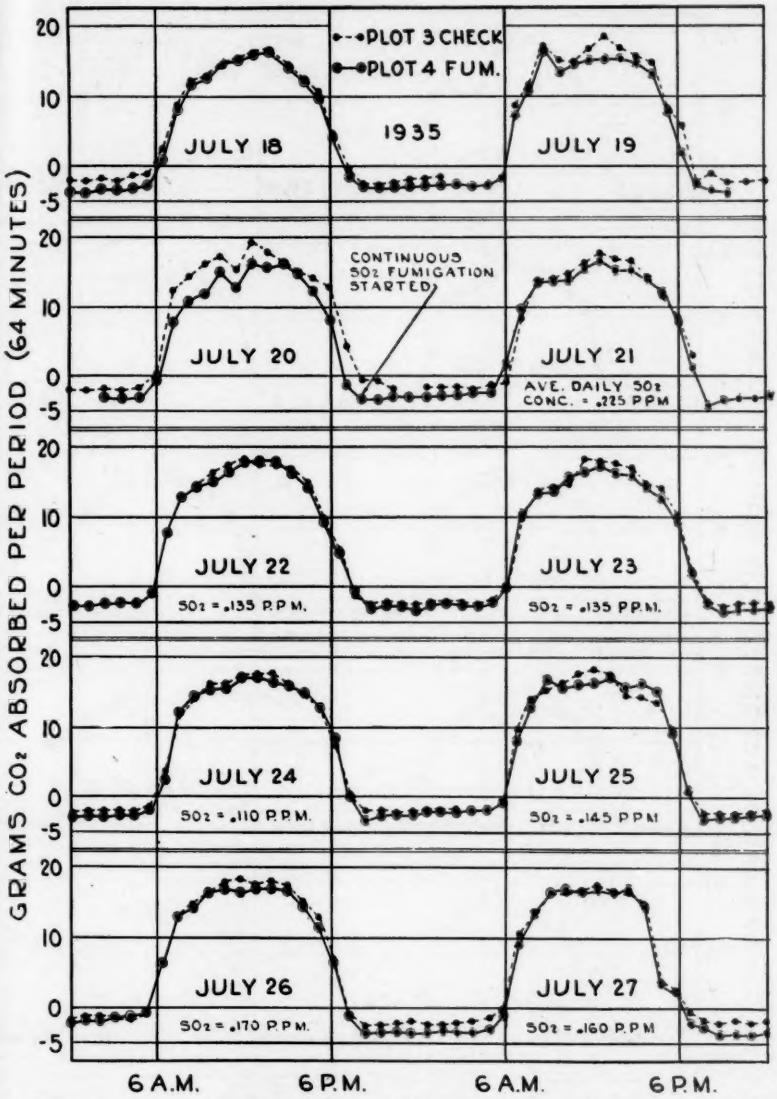

FIG. 12. Chart A, July 18–27, 1935. Apparent assimilation and respiration of plots 3 and 4 at Logan, Utah. Plot 4 was fumigated continuously from July 20 to September 3 (45 days) with 0.14 p.p.m. SO_2.

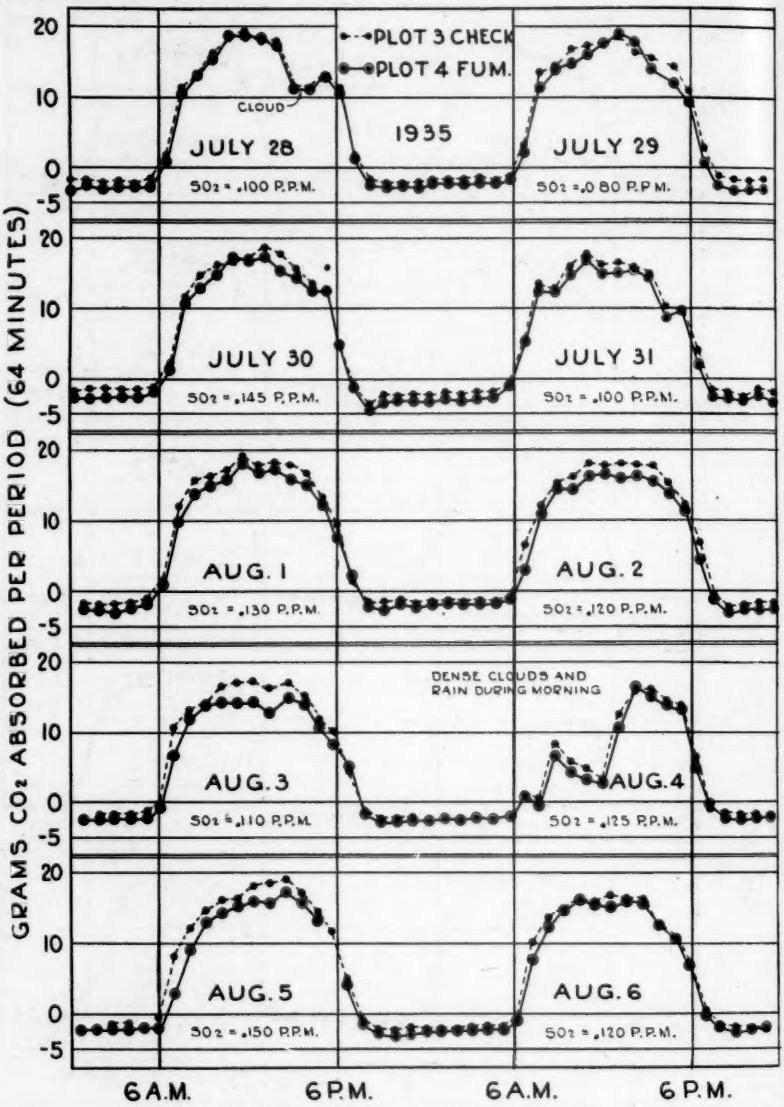

FIG. 12. Chart B, July 28–August 6, 1935.

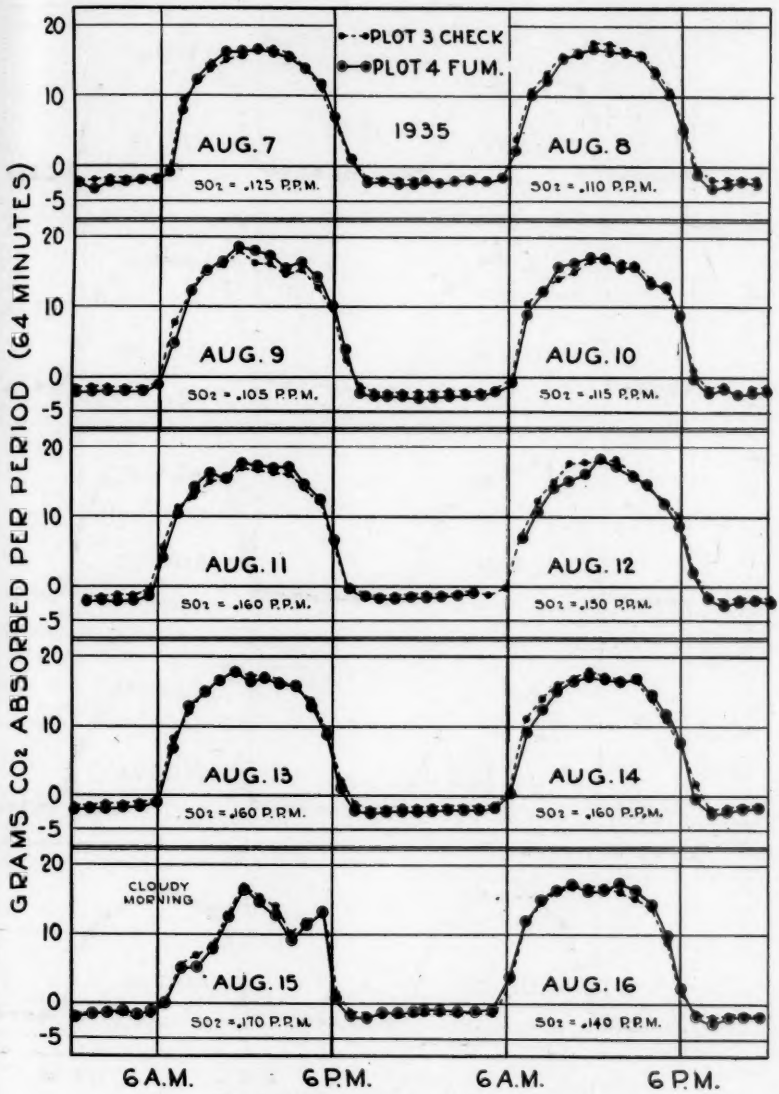

FIG. 12. Chart C, August 7–16, 1935.

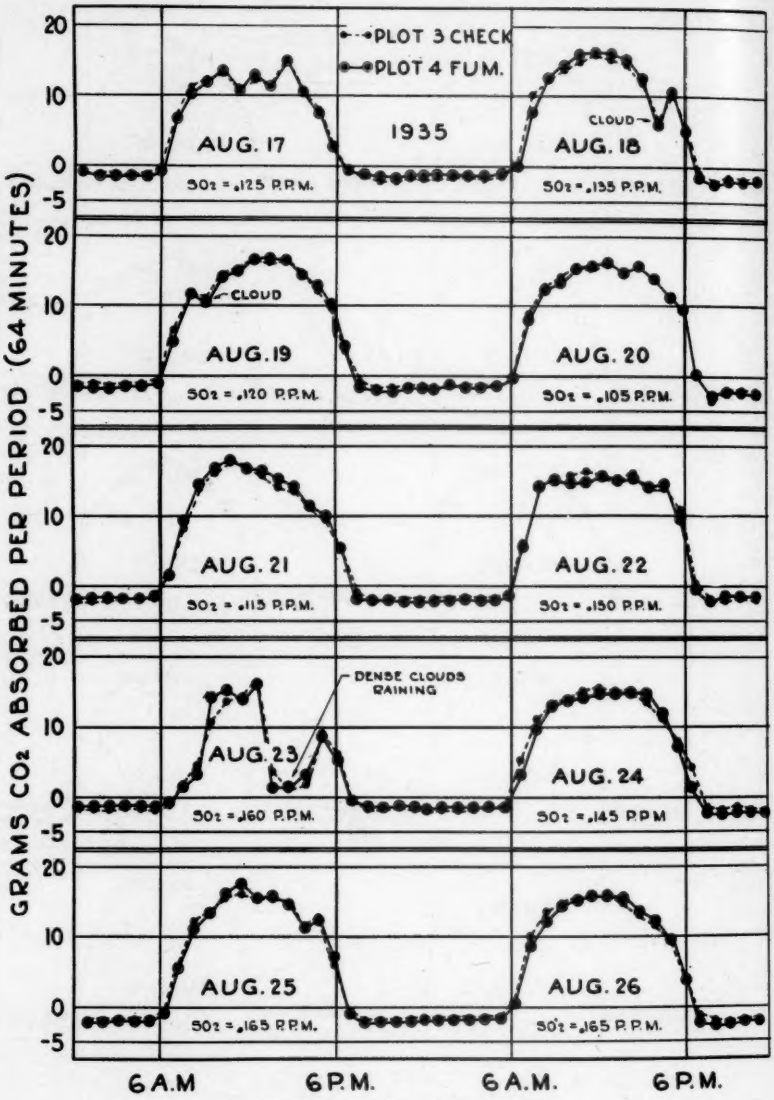

FIG. 12. Chart D, August 17–26, 1935.

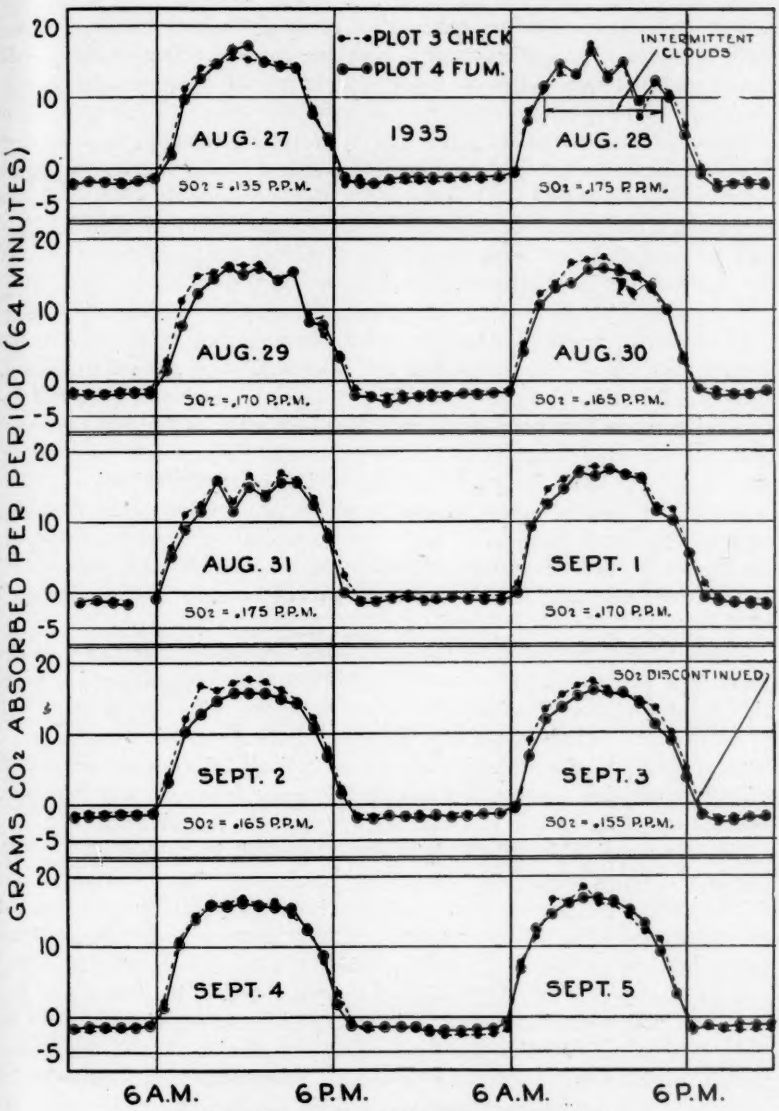

FIG. 12. Chart E, August 27–September 5, 1935.

it had been before the fumigation started. Then for 8 days the rate of apparent assimilation of the treated plot fell off about 7 per cent. owing, we believe, to failure of irrigation water to penetrate the plot. During this period the treated plot seemed to have a yellowish appearance, as noted by several observers, and it was estimated that its color was about 10 per cent. more yellow than the check plot. After a heavy irrigation of the plots on August 3, the color of plot 4 seemed to improve, so that a few days later no differences could be observed. After August 6, the curves of apparent assimilation showed an increase of 2 per cent., and the net assimilation an increase of 6 per cent. and 8 per cent., suggesting a stimulation of photosynthesis by the sulphur dioxide treatment. During the last 6 days of the fumigation, owing possibly to an increased concentration of sulphur dioxide, which averaged about 0.17 p.p.m. during this period, the apparent assimilation of the treated plot fell off 4 per cent., and the net assimilation receded to normal. Finally, after the fumigation was discontinued, the curve of apparent assimilation of the fumigated plot for 2 days before the plots were harvested was 98 per cent. of the check, while the net assimilation was 107 per cent. of the check. It should be noted that the total amount of respiration of plot 4 was 26 per cent. greater than that of plot 3, but the difference decreased as the experiment proceeded. This circumstance favors, perhaps unduly, the treated plot in the adjusted ratios of the net assimilation. This difference of respiration may have been caused by a difference in "soil respiration" of the two plots already referred to.

This detailed record of the carbon dioxide exchange, together with the yield data, would seem to be conclusive evidence that this prolonged fumigation at this low concentration produced no deleterious effect upon the growth of alfalfa, and for about half the time a definite stimulation is suggested. The reduced rate of assimilation which was indicated during the last 6 days of the fumigation represents a decrease of only 1 per cent. of the total assimilation of the treated plot over the period of the experiment.

Sulphur and chlorophyll data are presented in table XXV. The amounts of chlorophyll were nearly identical in plots 3 and 4 after August 6. Unfortunately, preparations had not been made earlier to carry out these analyses when differences possibly may have existed. The sulphur determinations showed a regular increase in the sulphur content of the leaves and stems of plot 4, but the maximum amount found in the leaves was 1.52 per cent., which was insufficient to interfere with the functions of the leaf. Table XXIII indicates that 30.94 gm. of sulphur dioxide were absorbed from the air, equivalent to 15.47 gm. of sulphur. Only 6.937 gm. of this amount was recovered in the plant tissue (table XXV). As was noted above, part of this sulphur appeared on the second 1936 crop, when more sulphur was found in the top growth than was added by the fumigation treatments.

TABLE XXIV

BALANCE SHEET OF CARBON DIOXIDE EXCHANGE AND GROWTH OF ALFALFA PLOTS 3 AND 4—1935

DATE (1935)	TOTAL APPARENT ASSIMILATION CO_2				NIGHT RESPIRATION CO_2				NET ASSIMILATION CO_2			
	PLOT 3 CHECK	PLOT 4 FUMIGATED	RATIO 4/3 ACTUAL	RATIO 4/3 ADJUSTED	PLOT 3 CHECK	PLOT 4 FUMIGATED	RATIO 4/3 ACTUAL	RATIO 4/3 ADJUSTED	PLOT 3 CHECK	PLOT 4 FUMIGATED	RATIO 4/3 ACTUAL	RATIO 4/3 ADJUSTED
	gm.	*gm.*	%	%	*gm.*	*gm.*	%	%	*gm.*	*gm.*	%	%
Before fumigation												
7/17– 7/20	609	541	89	92	69	121	175	126	540	420	78	86
7/18– 7/19	304	287	95	98	36	56	156	112	268	231	86	95
Continuous fumigation started at 8: 40 P.M. July 20—average SO_2 concentration 0.141 p.p.m.												
7/21– 7/28	1300	1260	97	100	157	218	139	100	1143	1042	91	100
7/29– 8/ 6	1415	1271	90	93	157	225	143	103	1258	1046	83	91
8/ 7– 8/16	1525	1507	99	102	159	198	125	90	1366	1309	96	106
8/17– 8/26	1337	1325	99	102	179	186	104	75	1158	1139	98	108
8/27– 9/ 2	993	929	93	96	107	123	115	83	886	806	91	100
Fumigation discontinued at 6: 45 P.M., September 3												
9/ 3– 9/ 5	439	419	95	98	61	51	84	61	378	368	97	107
Totals	7618	7252	95	98	889	1122	126	91	6729	6130	91	100

Equivalent dry matter (44 per cent. carbon) (grams)			4171	3799
Functioning tissue	Plot 3	Plot 4		
Interim samples for analysis	105[a]	103[a]		
	199[b]	200[b]		
Harvest September 6	483[a]	460[a]		
	860[b]	963[b]		
Dead tissue September 6	135	227		
Total tissue to September 6			1782	1953
Dry matter July 17 (estimated from comparable alfalfa harvested on July 17) (grams)			737	762
Top growth during experiment (grams)			1045	1191
Top growth—percentage of total CO_2 assimilated			25.0	31.4
Probable root increment (grams)			3126	2608

[a] Leaves. [b] Stems.

TABLE XXV

ANALYSES OF PLOTS 3 AND 4 FOR SULPHUR AND CHLOROPHYLL—1935

DATE (1935)	PLANT TISSUE	SULPHUR (S)						CHLOROPHYLL		
		PLOT 3 (CHECK)			PLOT 4 (FUMIGATED)			PLOT 3 CHECK	PLOT 4 FUMIGATED	OUTSIDE* CHECK
		WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)	WEIGHT OF TISSUE	SULPHUR (S)	WEIGHT (S)			
		gm.	%	*gm.*	*gm.*	%	*gm.*	%	%	%
July 18	Leaves	4.6	0.432	0.020	8.5	0.429	0.036	.....	.....	.....
" 27	"	13.5	0.464	0.063	15.3	0.952	0.146	.....	.....	.....
August 2	"	21.3	0.448	0.095	19.6	1.027	0.201	.....	.....	.....
" 6	"	18.6	0.438	0.081	17.0	1.240	0.211	1.47	1.46	.....
" 16	"	32.7	0.441	0.144	30.0	1.475	0.443	1.31	1.27	1.09
September 6	"	483	0.480	2.318	460	1.520	6.992	1.29	1.29	1.16
" 6	Dead leaves	44	0.380	0.167	51	0.882	0.450	.....	.....	.....
July 18	Stems	6.5	0.163	0.011	11.1	0.151	0.017	.....	.....	.....
" 27	"	24.1	0.130	0.031	28.3	0.140	0.040	.....	.....	.....
August 2	"	40.2	0.136	0.055	37.9	0.163	0.062	.....	.....	.....
" 6	"	35.1	0.171	0.060	34.7	0.177	0.061	.....	.....	.....
" 16	"	64.4	0.135	0.087	60.7	0.225	0.137	.....	.....	.....
September 6	"	860	0.166	1.428	963	0.257	2.475	.....	.....	.....
" 6	Dead stems	100	0.160[a]	0.160	193	0.200[a]	0.386	.....	.....	.....
" 6	Leaves and stems	.....	0.278	.....	.....	0.665	.....	.....	.....	.....
	Total			4.720			11.657			
	Excess of S in plot 4 over plot 3						6.937			

* Comparable alfalfa grown adjacent to and outside of plant chambers.
[a] Estimated.

Evidently there was some translocation of the sulphur to the roots in this 1935 experiment.

Finally, it may be pointed out that the "rate of absorption" of sulphur dioxide in this series of experiments, as shown in the tables of fumigation data, is a valuable index to the effectiveness of the treatments in affecting photosynthesis. There is a gradual lowering of this rate of sulphur dioxide absorption in the series, until in the last experiment, the gas could be oxidized as rapidly as it was absorbed, so that it had no effect in reducing photosynthesis over a very protracted period.

Discussion

The experiments described in this paper were undertaken primarily to find out if concentrations of sulphur dioxide which did not produce any visible injury to the plant, whether applied as high concentrations of short duration, as low intermittent concentrations, or as continuous fumigations throughout the life of the plant, nevertheless could cause injury to the protoplasm or to any plant structure. In other words, it was the purpose to find out whether "invisible injury" really existed and, if it did exist, how far-reaching an effect it could have.

The experiments have shown definitely that sulphur dioxide can produce reductions—the amount depending on the intensity of the fumigation—in the rate of photosynthesis during the actual duration of the fumigations, and sometimes for a number of hours thereafter, particularly in cases in which considerable sulphur dioxide previously has been absorbed, without producing any visible effect on the leaves. As soon as the fumigations were over, however, the rate of photosynthesis returned more or less quickly to normal. The effect upon photosynthesis is not dissimilar to that produced by a passing cloud, except that the cloud shadow usually produces an effect several times larger. The return to normal is frequently as rapid as the return after a cloud passes. Though there is a reduction in rate in both cases, the complete return afterward is definite indication that no injury has occurred in either case. Since the rate of photosynthesis is so delicate a yardstick, capable of registering in a definite way even the slightest effect upon any of the functions of the plant, it is hard to conceive of any injury which would not be clearly reflected by a more or less permanent change in the photosynthetic level.

Though the attempt has been made many times, and in many ways, to apply sulphur dioxide to plants in such a manner as to reduce the yield of the crop without producing visible injury, this has not been accomplished in any of our experiments. Further confirmation for wheat was shown by SWAIN and JOHNSON (**4**), who found no interference with reproduction processes and no reduction in the yield of that grain, as a result of fumiga-

tions with sulphur dioxide for several hours daily in concentrations too low to cause visible markings.

Many toxic substances are known to produce a stimulation in plant activity when applied in subtoxic quantities. Sulphur dioxide seems to be no exception to this rule. In addition, sulphur dioxide is rapidly oxidized to the sulphate form by the plant, which is the form in which plants use sulphur in their nutrition.

Just what percentage of sulphur in plant tissues is optimum for plant growth has not been determined. These experiments have shown that it can vary through a wide range, certainly from 0.5 per cent. to 1.5 per cent. in alfalfa leaves, without affecting the photosynthetic level. As 2 per cent. sulphur is approached, alfalfa leaves begin to show chlorotic markings, which rapidly increase as the percentage of sulphur increases.

Summary

1. Nine fumigation experiments with alfalfa plots and their corresponding checks are described, in which the treatments range from fumigations which produced almost complete foliar destruction, to long-continued sublethal dosages of the gas. The effects of the treatments have been determined by measuring the rate of photosynthesis and respiration of the plot in comparison with a similar check plot extending for a period from several days before the fumigations were started to several days after they were discontinued. Yield data confirm the carbon dioxide exchange data. The observations on the effects of the fumigations on the plants have been supplemented by chemical analyses of the leaves and stems for sulphur and chlorophyll.

2. Extensive foliar destruction of alfalfa by a sulphur dioxide fumigation is followed by a rather rapid growth of new leaves and a corresponding re-establishment of photosynthetic activity. In one experiment the latter was restored to about 75 per cent. of normal in 9 days following a fumigation which caused 90 to 95 per cent. of leaf destruction. In another similar experiment 65 per cent. recovery of photosynthesis occurred in 10 days, and 80 per cent. in 15 days.

3. Short fumigations with high concentrations of sulphur dioxide which were discontinued before an appreciable amount of leaf destruction was produced, caused large reductions in the rate of photosynthesis during the fumigation, but the photosynthesis began to increase within an hour after the treatment. The effect of such fumigations could be observed in a somewhat lowered rate of apparent assimilation for about 2 days after the treatment. Thereafter the rate was normal or greater than normal. The effect of two such treatments on the yield of a single crop of alfalfa, as calculated from the carbon dioxide exchange data, agrees with the decrease in yield calculated from a yield-leaf destruction equation previously published.

4. A number of short fumigations, with concentrations ranging from 0.7 to 1.26 p.p.m., each caused a definite decrease in the photosynthetic level; but immediately thereafter the activity rose to a normal or greater than normal level, so that when the total assimilation was calculated the decreased assimilation that had occurred during the fumigation was largely counterbalanced by increased assimilation after the fumigation was discontinued. The net effect of the fumigations was, therefore, practically zero. The results suggest that subsequent to such fumigations there was a slight stimulation of assimilation.

5. A plot was fumigated for 4 hours on each of 3 successive mornings with about 0.6 p.p.m. After several days this treatment was repeated. During the time each fumigation was in progress the assimilation level fell to about 75 per cent. of the check, but returned practically to normal in the afternoon. The 6 fumigations caused a decrease in net assimilation of about 10 per cent. of the total assimilation during the 16 days of observation or about 5 per cent. of the net assimilation of a full-crop period.

6. Another plot was fumigated on 5 successive mornings with about 0.43 p.p.m. Then after 5 days this treatment was repeated. During each of these fumigations the average level of apparent assimilation was reduced to 88 per cent. of the check, but the level returned to a normal or greater than normal level as soon as the fumigation was discontinued. Again, the reduced rate of apparent assimilation during the fumigation periods was largely counterbalanced by increased assimilation subsequent to the fumigations, so that a significant reduction in the total amount of assimilation during the experiment probably did not occur.

7. A plot was fumigated on 31 days, 27 of them in the morning, with 0.35 p.p.m. for 4.25 hours each day. In addition to the fumigation treatments, water was withheld from the plots for 3 weeks so that at the end of this period temporary wilting occurred on both plots during the hot part of 3 days. The average effect of these fumigations was to reduce the assimilation level about 6 per cent. during the progress of the fumigation, but immediately after the fumigation was discontinued the assimilation level practically returned to normal. The drought did not modify the effect of the fumigation, as shown by the relative photosynthetic activity of the two plots in the morning and afternoon hours, even on the three days when wilting occurred.

8. Three long continuous fumigations in 1935 showed that no effect of the fumigation occurred with 0.24 p.p.m. for about 3 days, with 0.19 p.p.m. for 11 days, and with 0.14 p.p.m. for at least 39 days. The data even suggest a slight stimulation of apparent assimilation during these periods. The continued application of the higher concentrations caused a gradual reduction in the photosynthetic level of the treated plot, with attendant visible

markings of the leaves and premature shedding of the older leaves. The reduced rates of photosynthesis were paralleled by reduced amounts of functioning leaf tissue.

9. Chlorophyll analyses in these experiments showed a definite tendency for the longer fumigations to reduce slightly the chlorophyll content in comparison with that of the check plot, but comparable alfalfa growing outside of the plant chambers usually had less chlorophyll than the treated plot. The reduction in the chlorophyll content of the treated plots was probably without practical significance in every case, except the 2 long-continued 1935 fumigations with the higher concentrations of sulphur dioxide, which produced appreciable visible markings. The chlorophyll content of the plot which received 0.14 p.p.m. continuously was normal.

10. The sulphur analyses of the leaves and stems account for about 90 per cent. of the sulphur dioxide added in the short fumigations. The remainder was probably absorbed on the walls of the plant chamber and in the soil. The continuous fumigations of 1935 exhibited a large deficit of sulphur in the plant tissue as compared with the sulphur dioxide added. It was shown that most of this deficit in one experiment was transferred to the roots and could be accounted for in the crops of 1936.

11. Absorption by the soil was too slight to be determined analytically. Fourteen times as much sulphate was found in the soil adhering to the roots as in the soil mass, suggesting a passage outward of sulphate from the roots to the soil.

12. The rate of absorption of the gas by the leaves decreased in this series of fumigations along with decreasing effects on photosynthesis of the fumigation treatments.

13. Leaf analyses showed large increments of sulphur in the protracted fumigations. When the sulphur content of the leaf approached 2 per cent., the leaf became chlorotic and soon ceased to function. At about 1.5 per cent. to 1.7 per cent. or less, the leaf appeared to be normal.

14. The complete return to its normal level of photosynthesis, following fumigations which did not produce visible injury, would indicate that in such fumigations there is no "invisible injury," either to the protoplasm or other plant structure.

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INFLUENCE OF WEAK ELECTRIC CURRENTS UPON THE GROWTH OF THE COLEOPTILE

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(with three figures)

Introduction

Among the numerous contributions to the electrophysiology of the plant there were until lately no investigations which dealt with the problem of the relation between bioelectrical phenomena and growth, the main reason being the absence of a satisfactory theory. The purely empirical data, which have been obtained by various authors, could not attract the attention of physiologists to this problem. Interest in electrophysiological investigations of growth has increased lately, since the studies of the growth hormone have thrown a new light upon this phenomenon. The first stimulus in this direction was given as a result of the ideas developed by one of the writers (**3**) on the effect of electrophysiological polarization upon the translocation of the growth hormone in organs of higher plants. When one compares the data of Bose, Brauner, Waller, and other physiologists on the effect of geo- and photoinduction on changes in the electric potentials of various parts of the plant organism, it is easy to notice that growth is always taking place in such a way that the concentration of the growth hormone increases in the positively charged regions and diminishes in those regions where, according to electrometric observations, the plant has a negative charge. On the basis of this comparison the conclusion can be drawn that in organs, which are subjected to the influence of photo- and geoinduction, the growth hormone is transferred in the direction of the increasing potential.

In further development of this idea Went (**12**) has made the assumption that, in general, the translocation of the growth hormone, in organs of the plant from places where it is produced to the growing zone and beyond, depends on the distribution of the electrical potential. Went showed that the basic dyes diffuse in sections of the stalk of *Impatiens balsamina* in the direction of the apex, while the acid dyes diffuse toward the basal part, which, according to Went, always has a positive charge. Auxin, as determined by Kögl (**8**), is an acid. Therefore Went considers that the anion of this acid, which is its physiologically active part, must move in the stalk in the same direction as the acid dyes do, *i.e.*, from the apex to the base. However, one does not find in Went's work any experiments which would directly confirm this assumption about the translocation of the growth hormone.

In 1933, KÖGL (**8**) described experiments which were based on the observation that in various hours of the day the decapitated coleoptiles of oats react to the same dose of auxin by curvatures of different intensity (when the experiments were carried out according to the method of WENT, **11**). Searching for the cause of this phenomenon KÖGL came to the idea that the effect of atmospheric electricity, namely, the ionization of the air, might be the cause of these differences. This idea seems to be confirmed by the fact that when coleoptiles are placed in zinc or tin containers they show a considerably greater "sensitivity" and the fluctuations in the intensity of the curvatures during the day are far less than in cases where the experiments are carried out in the ordinary way. On the assumption that the cause of the observed changes in sensitivity of the coleoptiles is the passing over the plant of an electric current of very low intensity, KÖGL carried out the following experiments. A very weak electric current of approximately 0.0008 milliampere was passed through decapitated coleoptiles of oats, to one side of which there was attached a small cube of agar-agar containing auxin. One of the electrodes—a moistened silk thread—was attached to the agar cube, while the other electrode was connected with the basal portion of the coleoptile. The results of this experiment showed that when the agar cube was connected with the negative pole, the coleoptiles reacted to the same quantity of growth hormone by more vigorous bending than when the cube was connected with the positive pole. The ratio between the intensities of the cuvatures was 12 : 1. KÖGL explained these results by WENT'S scheme, assuming that in case of the first contact "the transport of the physiologically active auxin anions to the base of the coleoptile is accelerated," while in the other case it is retarded.

In 1934 KOCH (**7**), of GUTTENBERG'S laboratory, published a paper, in which a whole chapter is devoted to the problem of translocation of the growth hormone in coleoptiles of oats and also in the hypocotyls and roots of sunflower and some other plants placed in the electric field, when an electric current passed through these organs. The starting point of the first group of experiments of this author were the investigations of BRAUNER and BÜNNING (**2**), which showed that the coleoptiles of oats bend toward the negative pole when placed in an electric field of high intensity, between two metallic plates attached to the poles of a battery. On the other hand, the root of the broad bean (*Vicia faba*) under the same conditions bends in the opposite direction. The authors explain this phenomenon as being due to the deviation of the growth hormone flow in the direction of that part of the organ, which became positively charged due to induction. HARTMANN (**6**) repeated these experiments, but obtained different results. On the basis of this work the author came to the conclusion that the influence of the electric field upon the growth of the plant is a more complicated

phenomenon and that it cannot be explained by the translocation of the growth hormone to the positively charged side of the organ.

KOCH attempted to clear up this contradiction, but unfortunately he carried out his experiments in such a way that they cannot be compared with the BRAUNER-BÜNNING and the HARTMANN experiments. Instead of placing the plant between the electrodes in atmospheric air, as previous authors did, he immersed them in water into which there were also immersed the electrodes in the form of platinum plates attached to the poles of a flashlight battery, which gave a current of 0.2 ampere at a tension of 4.4 volts. It is clear that under such conditions KOCH's "electric field" created not an induction of + and – charges on the opposite ends of the plant organism, but the passage through it of a current of unknown strength, but which was less than 0.2 ampere. KOCH assumes that the phenomenon of induction should take place in the coleoptile of oats even when it is immersed in water, since the cuticle of this organ, according to the author, acts as an insulator. The experiments, however, show that the cuticle of the coleoptile as well as of other plant organs, especially in the wet state, exercises a very small resistance to the electric current. Therefore, under the conditions of KOCH's experiments, the current was passing in the plant in the same direction as in the surrounding water.

Of much greater interest are KOCH's experiments in which he is trying to determine whether the growth hormone of oats, under the influence of an electric current, really moves in agar-agar to the positive pole, as it should, according to WENT'S theory of polarization. KOCH placed a certain quantity of coleoptile tips on an agar plate which was 1–1.5 cm. in length and 0.5 cm. in width. At the sides of this plate he placed platinum electrodes and passed a current from a flashlight battery (4.4 volts). Then the author cut out cubes from various parts of the plate, put them unilaterally upon the decapitated coleoptiles of oats and after a definite period measured the curvatures of the coleoptiles. The greatest curvatures were formed when cubes which were cut out near the positive pole were used. Cubes taken from the opposite part of the agar plate (at the negative pole) did not cause any bending. On the basis of these results the author concludes that under the influence of the electric current the growth substance moves in agar toward the positive pole.

It is easy to show, however, that these experiments are not definite. When an electric current passes through an agar plate the phenomenon of electrolysis is undoubtedly taking place in it and this causes the accumulation of the products of hydrolysis at the electrodes in the agar plate. Basic products of electrolysis at the negative pole, uniting with the growth hormone which is an acid, will transform it into an inactive state of a salt. It is, therefore, not known to what the results of KOCH's experiments have to

be ascribed: whether to the translocation of the hormone to the positive electrode, to its inactivation at the negative electrode, or to the combined activity of these two factors.

The third series of KOCH's experiments likewise is not very convincing. These were carried out on young hypocotyls of *Helianthus* and *Lupinus*. The author inserted the positive electrode into the side of the upper part of the hypocotyl to a depth of about 1 mm.; the negative electrode was inserted into the opposite side of the hypocotyl, 2 cm. lower than the positive one. An electric current from a flashlight battery was passed through the organ for 30 minutes. Two hours later definite curvatures had formed in the direction away from the positive pole. This phenomenon, according to KOCH, shows that the growth substance is attracted by the positive pole and thus an acceleration of growth on this side takes place. This conclusion, however, cannot be considered as established either. The cause of the acceleration of growth might have been a simple increase in acidity at the positive electrode due to electrolysis. STRUGGER (**10**), for instance, has shown recently that it is possible to accelerate the growth of cells at the growing zone (hypocotyl of the sunflower) with the aid of acid solutions of a definite pH. His results have been partially confirmed by BONNER (**1**), who considers that the acceleration of growth, by increasing the acidity, results from an activation of the growth hormone reserves: the inactive salt is changed to the active form of a free acid. Therefore, KOCH's experiments do not seem to prove the translocation of a growth hormone in the hypocotyl tissue to the positive pole.

Finally the work of RAMSHORN, of RUHLAND'S laboratory, has to be mentioned. In his electrophysiological investigations this author applied more perfect methods than KOCH and used electrodes which did not polarize. By determining the difference in the electric potentials between various points of the root, stalk, and leaves of young seedlings of the sunflower, oats, bean, and other plants with the aid of a quadrant electrometer, he showed that the curves of the electric potential distribution are very much like the curves of the rate of growth of various zones of these organs. The zones which show a more rapid growth are always electropositive as compared to those which grow slower. Changes in the difference of growth, which are connected with nutations, cause corresponding changes in potentials. Alterations in the difference of potentials may also be created by the introduction of the growth hormone in certain places of the organ. In this case again the zones which grow faster will become electropositive.

RAMSHORN studied also the effect of an outside electromotive force upon the growth of the hypocotyl of the sunflower and found that when the growing zone is connected with the positive pole an acceleration of growth of short duration may be observed. On the other hand, when it is connected

with the negative pole, then growth is retarded. Without going into details of other results of RAMSHORN's work, we will only mention his experiments on sections of the stalk of *Impatiens balsamina*, on which WENT carried out his studies. These experiments showed that the apical part of the stem is electropositive with respect to the basal one. On the basis of these results RAMSHORN came to the conclusion that the unequal diffusion of acid and basic dyes, which WENT observed on this object, cannot be explained by differences in electric potentials.

The brief review of the latest work in the electrophysiology of growth, which has been presented, shows that the conclusions of various workers in this field differ greatly even on purely experimental questions. As was shown in the review, the methods applied by some of the authors mentioned were not always free from criticism from the physical and physiological points of view. However, such preliminary work is necessary. In view of these considerations we believe it useful to publish briefly the main results of our electrophysiological studies, which were planned and partially carried out before the papers of KOCH and of RAMSHORN appeared, and with which our work has some common points.

The starting point of our investigations were KÖGL's experiments mentioned above. If an electrical current of 10^{-6} to 10^{-7} amperes really accelerates or retards, depending on the direction of the current, the translocation of the growth hormone in the decapitated coleoptile, it may be assumed that the same phenomenon should also take place in the undecapitated coleoptile which is obtaining the growth substance from its own top, namely, that the rate of growth would change under the influence of the growth hormone depending upon the direction of the electromotive force which is applied to it. If the WENT-KÖGL scheme is correct, then the current, which is passing from the base to the apex of the coleoptile, should cause a certain acceleration in growth while the current, which is directed to the opposite side, should retard growth. The main object of our experiments was to verify this conclusion.

Methods

First we will describe the method used. In all our investigations we applied only such electrodes as do not polarize and which were described previously by one of the writers (**5**). As will be seen from figure 1, such an electrode is made of two communicating glass tubes, *A* and *B*. The wider tube, *A* (about 7 mm. in diameter and 60 mm. long), is filled with a saturated solution of $ZnSO_4$, while the narrow one, *B* (2 mm. in diameter), is filled with tap water. A kaolin stopper, *a*, which is previously soaked in water, and 2 layers of cotton, *b*, separate the water in the capillary tube, *B*, from the zinc sulphate solution in *A*. An amalgamated rod, *d*, with a sold-

ered clamp, *e*, is put into the tube, *A*, through a rubber stopper, *f*. A silk thread, *i*, of which one end is immersed in water, is connected by its other end with the object under investigation.

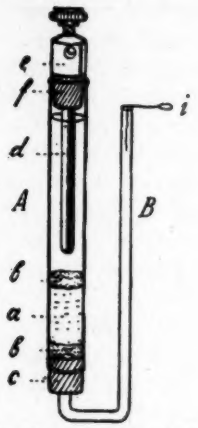

FIG. 1. Electrode used in the work reported.

Figure 2 shows how the two electrodes are fastened to the stand, which is made of a paraffined cork and glass rods (**5**). This figure also shows how the electrodes are connected with the coleoptile, which was at a certain time before the experiment cut off from the mesocotyl and freed from the primordial leaf (**4**). The coleoptile is placed by its lower end into a cut of the cork plate, *a*, which is in turn attached by a nickel pin to a paraffin layer in a cylindrical glass container, *c*.

As was mentioned above, both electrodes were connected with the coleoptile through silk threads. One of these threads had on the end a loop of about 0.5 mm. in diameter. This loop was set before the beginning of the experiment on the top of the coleoptile (fig. 2). The other electrode, also through silk thread, was connected with the water, which was on the paraffin layer in the container, *c*, and into which the coleoptile was immersed by its lower end. It must also be mentioned that the electrodes were always arranged in such a way that the open ends of the capillaries, *B*, which are filled with water, were on the same horizontal plane with the point of attachment of the silk threads to the object (fig. 2). Thus we avoided the formation of a siphon and the running over of the water from the tubes of the electrodes to the object, or in the opposite direction over the threads.

The stand with the electrodes and with the plant was covered by a glass bell jar throughout the experiment. A well-moistened sponge was kept under the bell jar. The experiments were carried out in a dark room, the

temperature of which changed but little. The temperature fluctuations during the course of our experiments usually did not exceed 0.1 to 0.2° C. Growth was measured by an horizontal microscope with an ocular micrometer, each division of which was equal to 50 μ. The measurements and all the other manipulations were made by the light of a red photographic lamp.

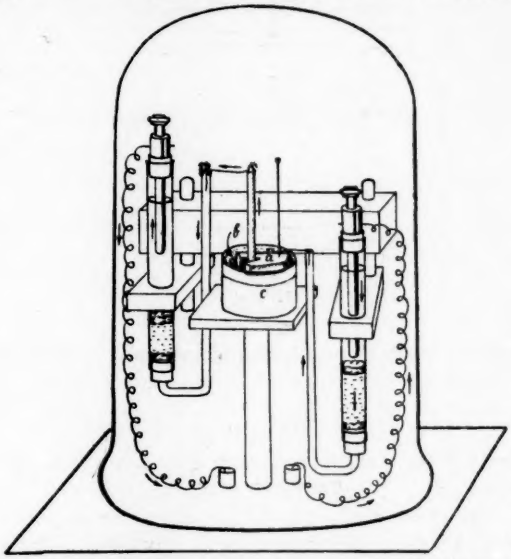

FIG. 2. Electrodes attached to a stand, showing method of making contacts with the coleoptile.

The source of the electric current was an accumulator of 4 volts connected with a rheostat of 120 ohms. The rheostat had a moving contact pointer, which enabled the introduction into the plant of a current of the desired strength through the side connection. The current strength was measured by a galvanometer the sensitivity of which was $0.14 \cdot 10^{-6}$ amperes. The direction of the current could be reversed by a reversible key. All this arrangement is schematically represented in figure 3.

The main object of our investigation was the isolated coleoptiles of oats (*Avena sativa* var. Siegeshafer Svalöf), which was from 20 to 30 mm. in length. We worked also with the coleoptile of whole uninjured oats seedlings. Several experiments were carried out with rye (*Secale cereale*).

Experimentation

ISOLATED COLEOPTILES

The following tables (I–XX) give the results of experiments with isolated coleoptiles. The arrow pointing upward shows that the current ran

from the base of the coleoptile to the apex. The current running in the opposite direction is indicated by arrows pointing downward. Growth is given everywhere in divisions of the scale of the ocular micrometer (50 μ).

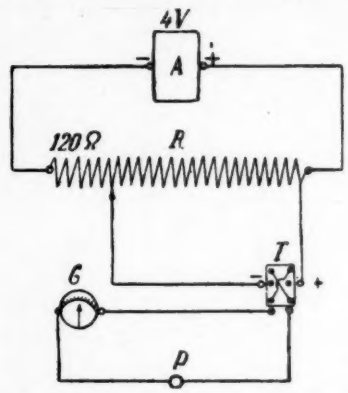

FIG. 3. Diagram of circuits used in making the measurements recorded.

TABLE I

EXPERIMENT, MAY 8, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 4. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		1:20 P.M.		
2	0		1:40 "	3.5	
3	0		2:00 "	3.5	Average 3.5
4	0		2:20 "	4.5	
5	0*		2:40 "	3.0	
6	$1.13 \cdot 10^{-6}$	↑	3:00 "	**5.5**	
7	"	↑	3:20 "	4.5	
8	"	↑	3:40 "	3.5	Average 3.1
9	"	↑	4:00 "	3.0	
10	"	↑	4:20 "	1.5	
11	"	↑	4:40 "	1.0	

* Current on.

TABLE II

EXPERIMENT, MAY 9, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 5. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		11: 20 A.M.		
2	0		11: 40 "	3.25	
3	0		12: 00 M.	2.75	Average 3.25
4	0		12: 20 P.M.	3.25	
5	0*		12: 40 "	3.75	
6	$1.88 \cdot 10^{-6}$	↑	1: 00 "	**5.0**	
7	"	↑	1: 20 "	4.25	
8	"	↑	1: 40 "	3.25	Average 3.0
9	"	↑	2: 00 "	2.5	
10	"	↑	2: 20 "	2.0	

* Current on.

TABLE III

EXPERIMENT, MAY 21, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 17. TEMPERATURE 22° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		7: 20 A.M.		
2	0		7: 40 "	3.5	
3	0		8: 00 "	3.5	Average 3.5
4	0*		8: 20 "	3.5	
5	$0.5 \cdot 10^{-6}$	↑	8: 40 "	**4.5**	
6	"	↑	9: 00 "	4.25	
7	"	↑	9: 20 "	3.25	Average 3.5
8	"	↑	9: 40 "	3.0	

* Current on.

TABLE IV

EXPERIMENT, DECEMBER 5, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON NOVEMBER 29. TEMPERATURE 14.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		2:40 P.M.		
2	0		3:00 "	3.0	
3	0		3:20 "	3.0	Average 3.0
4	0		3:40 "	3.0	
5	0*		4:00 "	3.0	
6	$0.5 \cdot 10^{-6}$	↑	4:20 "	**4.0**	
7	"	↑	4:40 "	3.0	
8	"	↑	5:00 "	3.0	Average 2.8
9	"	↑	5:20 "	2.5	

* Current on.

TABLE V

EXPERIMENT, FEBRUARY 7, 1935. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON FEBRUARY 1. LENGTH OF COLEOPTILE 23 MM. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		1:40 P.M.		
2	0		2:00 "	1.0	
3	0		2:20 "	1.5	
4	0		2:40 "	2.5	Average 2.2
5	0*		3:00 "	2.5	
6	$0.4 \cdot 10^{-6}$	↑	3:20 "	**4.0**	
7	"	↑	3:40 "	3.25	
8	"	↑	4:00 "	2.75	Average 2.7
9	"	↑	4:20 "	2.0	

* Current on.

TABLE VI

EXPERIMENT, DECEMBER 19, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 13. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		6: 20 P.M.	
2	0		6: 40 "	1.5
3	0		7: 00 "	1.0
4	0*		7: 20 "	1.0
5	$0.3 \cdot 10^{-6}$	↑	7: 40 "	**2.5**
6	"	↑	8: 00 "	**3.5**
7	"	↑	8: 20 "	2.5
8	"	↑	8: 40 "	1.5

* Current on.

TABLE VII

EXPERIMENT, DECEMBER 27, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 21. LENGTH OF COLEOPTILE 23 MM. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		5: 40 P.M.	
2	0		6: 00 "	1.0
3	0		6: 20 "	1.0
4	0*		6: 40 "	1.0
5	$0.3 \cdot 10^{-6}$	↑	7: 00 "	2.0
6	"	↑	7: 20 "	**2.75**
7	"	↑	7: 40 "	1.75
8	"	↑	8: 00 "	1.5

* Current on.

TABLE VIII

EXPERIMENT, DECEMBER 15, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 9. LENGTH OF COLEOPTILE 25 MM. TEMPERATURE 16.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		6:40 P.M.		
2	0		7:00 "	3.0	
3	0		7:20 "	2.0	
4	0		7:40 "	1.5	Average 1.7
5	0*		8:00 "	1.5	
6	$0.3 \cdot 10^{-6}$	↑	8:20 "	**4.0**	
7	"	↑	8:40 "	4.0	
8	"	↑	9:00 "	4.5	Average 4.3
9	"	↑	9:20 "	4.5	

* Current on.

TABLE IX

EXPERIMENT, JUNE 22, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON JUNE 17. LENGTH OF COLEOPTILE 24 MM. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		12:40 P.M.	
2	0		1:00 "	**3.0**
3	0		1:20 "	**4.5**
4	0		1:40 "	**4.0**
5	0*		2:00 "	**4.0**
6	$0.3 \cdot 10^{-6}$	↑	2:20 "	**6.5**
7	"	↑	2:40 "	**5.0†**
8	$0.6 \cdot 10^{-6}$	↑	3:00 "	**7.5**
9	"	↑	3:20 "	**4.5**

* Current on.

† Current strength was increased.

TABLE X

EXPERIMENT, JUNE 23, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON JUNE 19. LENGTH OF COLEOPTILE 25 MM. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	TIME OF OBSERVATION	DIRECTION OF CURRENT	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		12: 20 P.M.	
2	0		12: 40 "	3.0
3	0		1: 00 "	3.0
4	0		1: 20 "	4.5
5	0		1: 40 "	4.5
6	0*		2: 00 "	4.0
7	$0.3 \cdot 10^{-6}$	↑	2: 20 "	**6.0**
8	"	↑	2: 40 "	4.5†
9	$0.6 \cdot 10^{-6}$	↑	3: 00 "	5.25
10	"	↑	3: 20 "	5.25

* Current on.

† Current strength was increased.

The first 10 experiments (tables I to X), which were selected as typical from a great number of other similar ones, show that when a current of the order of 10^{-7} to 10^{-6} amperes is passing from the base to the apex through an isolated coleoptile of oats, a noticeable acceleration of growth is observed. This acceleration continues for quite a long time at relatively low temperatures (about 16° C.). At higher temperatures (about 20 to 22° C.), how-

TABLE XI

EXPERIMENT, MAY 13, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 10. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		1: 20 P.M.		
2	0		1: 40 "	2.25	
3	0		2: 00 "	2.75	
4	0		2: 20 "	4.0	
5	0		2: 40 "	3.5	Average 4.2
6	0*		3: 00 "	5.0	
7	$0.75 \cdot 10^{-6}$	↓	3: 20 "	**4.0**	
8	"	↓	3: 40 "	3.0	
9	"	↓	4: 00 "	3.0	Average 2.8
10	"	↓	4: 20 "	2.5	

* Current on.

ever, it is of a short duration and is sometimes accompanied by a slight decrease in the growth rate. Certain experiments (tables IX, X) show that an increase in the initial current strength during the experiment causes a new rise in the growth curve. This new rise is sometimes even greater than the initial one.

Let us now see how a current passing in the opposite direction, from the top to the base, affects growth of an isolated coleoptile.

TABLE XII

EXPERIMENT, JUNE 9, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON JUNE 5. LENGTH OF COLEOPTILE 27.5 MM. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		7:00 A.M.		
2	0		7:20 "	3.0	
3	0		7:40 "	2.75	
4	0		8:00 "	3.25	
5	0		8:20 "	3.0	
6	0		8:40 "	3.25	Average 3.1
7	0*		9:00 "	3.0	
8	$0.75 \cdot 10^{-6}$	↓	9:20 "	**3.0**	
9	"	↓	9:40 "	2.0	
10	"	↓	10:00 "	2.0	Average 2.0
11	"	↓	10:20 "	2.0	

* Current on.

TABLE XIII

EXPERIMENT, DECEMBER 10, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 4. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		2:00 P.M.		
2	0		2:20 "	2.0	
3	0		2:40 "	3.0	
4	0		3:00 "	3.0	Average 3.2
5	0*		3:20 "	3.5	
6	$0.3 \cdot 10^{-6}$	↓	3:40 "	**3.5**	
7	"	↓	4:00 "	2.0	
8	"	↓	4:20 "	2.0	Average 1.8
9	"	↓	4:40 "	1.5	
10	"	↓	5:00 "	1.5	

* Current on.

TABLE XIV

EXPERIMENT, DECEMBER 13, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 7. TEMPERATURE 16.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		5: 00 P.M.		
2	0		5: 20 "	1.5	
3	0		5: 40 "	1.5	
4	0		6: 00 "	2.0	Average 2.0
5	0*		6: 20 "	2.5	
6	$0.3 \cdot 10^{-6}$	↓	6: 40 "	**2.0**	
7	"	↓	7: 00 "	2.0	
8	"	↓	7: 20 "	2.0	Average 2.0
9	"	↓	7: 40 "	2.0	

* Current on.

TABLE XV

EXPERIMENT, DECEMBER 16, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 10. LENGTH OF COLEOPTILE 20 MM. TEMPERATURE 16° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		2: 00 P.M.		
2	0		2: 20 "	2.0	
3	0		2: 40 "	3.0	
4	0		3: 00 "	4.0	
5	0		3: 20 "	4.0	Average 3.7
6	0*		3: 40 "	3.5	
7	$0.3 \cdot 10^{-6}$	↓	4: 00 "	**3.0**	
8	"	↓	4: 20 "	2.0	
9	"	↓	4: 40 "	2.0	Average 2.0
10	"	↓	5: 00 "	2.0	

* Current on.

TABLE XVI

EXPERIMENT, DECEMBER 11, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON DECEMBER 5. TEMPERATURE 16.2° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		5:00 P.M.		
2	0		5:20 "	2.5	
3	0		5:40 "	3.0	
4	0		6:00 "	4.5	
5	0		6:20 "	3.5	
6	0		6:40 "	2.5	Average 3.1
7	0*		7:00 "	3.25	
8	$0.31 \cdot 10^{-6}$	↓	7:20 "	**2.5**	
9	"	↓	7:40 "	2.0	
10	"	↓	8:00 "	1.5	Average 1.5
11	"	↓	8:20 "	1.0	

* Current on.

TABLE XVII

EXPERIMENT, MAY 14, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 11. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		1:00 P.M.	
2	0		1:20 "	4.0
3	0		1:40 "	3.25
4	0*		2:00 "	3.0
5	$0.75 \cdot 10^{-6}$	↓	2:20 "	2.0
6	"	↓	2:40 "	1.5
7	"	↓	3:00 "	0.75
8	"	↓	3:20 "	1.0†
9	0		3:40 "	1.25
10	0		4:00 "	**2.75**
11	0		4:20 "	1.25
12	0		4:40 "	0.75
13	0		5:00 "	0.5

* Current on.

† Current switched off.

TABLE XVIII

EXPERIMENT, MAY 20, 1934. COLEOPTILE OF *AVENA SATIVA*. MATERIAL PLANTED ON MAY 16. TEMPERATURE 21.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		2: 00 P.M.	
2	0		2: 20 "	3.5
3	0		2: 40 "	4.5
4	0		3: 00 "	6.0
5	0*		3: 20 "	5.0
7	"	↓	4: 00 "	4.5
6	$0.56 \cdot 10^{-6}$	↓	3: 40 "	**4.5**
8	"	↓	4: 20 "	4.5†
9	0		4: 40 "	**3.25**
10	0		5: 00 "	4.0
11	0		5: 20 "	3.0

* Current on.

† Current switched off.

These eight experiments (tables XI–XVIII) are sufficient to show that a current of the same strength as in the first 10 experiments but passing in the opposite direction—from the top to the base—does not cause any increase in growth, but gives in most cases the opposite effect, namely, a retardation of growth of the coleoptile. This retardation, as is shown by two of the experiments (tables XVII, XVIII), lasts even after the current is switched off.

The coleoptiles of oats, even in their isolated state, are distinguished by an uneven and changeable growth rate, which makes the effect of external factors upon the growth less distinct. Consequently the writers considered it desirable to verify the results of the oats experiments by using another cereal which has a more uniform growth. As an example there are given here two experiments with rye (*Secale cereale* var. Petkus).

These experiments (tables XIX, XX) show that in this case the effect of the electric current upon growth of the coleoptile is the same as in the experiment with the oats. The current which passes from the base to the apex of the coleoptile causes a definite acceleration of growth of short duration. The current which passes in the opposite direction retards growth to a marked degree, and this retardation lasts for a long time even after the current is switched off.

UNINJURED SEEDLINGS

In addition to the experiments with the isolated coleoptiles described above, another series of experiments was carried on with uninjured seedlings

TABLE XIX

EXPERIMENT, MAY 17, 1934. COLEOPTILE OF *SECALE CEREALE*. AFTER REMOVAL OF THE PRIMORDIAL LEAF THE COLEOPTILE WAS KEPT IN A DARK ROOM FOR 1 HOUR AND 40 MINUTES. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		2:40 P.M.		
2	0		3:00 "	5.0	
3	0		3:20 "	4.5	
4	0		3:40 "	5.0	Average 4.8
5	0		4:00 "	4.5	
6	0*		4:20 "	5.0	
7	$0.6 \cdot 10^{-6}$	↑	4:40 "	**6.0**	
8	"	↑	5:00 "	6.0	
9	"	↑	5:20 "	5.5	
10	"	↑	5:40 "	4.5	Average 5.0
11	"	↑	6:00 "	4.5	
12	"	↑	6:20 "	4.5	
13	"	↑	6:40 "	4.0	
14	"	↑	7:00 "	4.0	

* Current on.

TABLE XX

EXPERIMENT, MAY 27, 1934. COLEOPTILE OF *SECALE CEREALE*. MATERIAL PLANTED ON MAY 23. LENGTH OF COLEOPTILE 34 MM. TEMPERATURE 20.5° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)	
	amp.				
1	0		5:40 P.M.		
2	0		6:00 "	5.5	
3	0		6:20 "	5.5	
4	0		6:40 "	7.0	Average 6.5
5	0*		7:00 "	7.0	
6	$0.56 \cdot 10^{-6}$	↓	7:20 "	**4.5**	
7	"	↓	7:40 "	4.5	
8	"	↓	8:00 "	4.0	Average 4.2
9	"†	↓	8:20 "	4.0	
10	0		8:40 "	2.25	
11	0		9:00 "	3.25	
12	0		9:20 "	3.25	

* Current on.

† Current switched off.

of oats, the roots of which were immersed in the water, while the lower part of the coleoptile was fastened in the cut of the cork plate, which covered the water container. The silk thread of the lower electrode was connected with the basal part of the coleoptile above the cork cover in some of the experiments (tables XXI–XXIV), while in other experiments (tables XXV–XXVII) it was immersed directly in the water so that the current passed not only through the coleoptile but also through the mesocotyl and the root system. In all other respects these experiments did not differ from the ones described above.

TABLE XXI

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 24° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		7: 00 A.M.	
2	0		7: 20 "	3.0
3	0		7: 40 "	6.0
4	0		8: 00 "	4.5
5	0		8: 20 "	4.5
6	0*		8: 40 "	4.0
7	$0.98 \cdot 10^{-6}$	↑	9: 00 "	**5.5**
8	"	↑	9: 20 "	4.5
9	"	↑	9: 40 "	3.5
10	"	↑	10: 00 "	3.0

* Current on.

TABLE XXII

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		12: 40 P.M.	
2	0		1: 00 "	4.0
3	0		1: 20 "	6.0
4	0		1: 40 "	5.5
5	0		2: 00 "	6.5
6	0*		2: 20 "	6.0
7	$0.7 \cdot 10^{-6}$	↑	2: 40 "	**7.0**
8	"	↑	3: 00 "	6.0
9	"	↑	3: 20 "	4.5

* Current on.

TABLE XXIII

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 24° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		7:00 A.M.	
2	0		7:20 "	2.0
3	0		7:40 "	4.5
4	0		8:00 "	3.75
5	0		8:20 "	3.0
6	0*		8:40 "	4.0
7	$0.98 \cdot 10^{-6}$	↓	9:00 "	**4.0**
8	"	↓	9:20 "	3.0
9	"	↓	9:40 "	2.5

* Current on.

TABLE XXIV

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 24° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		9:00 A.M.	
2	0		9:20 "	4.0
3	0		9:40 "	4.5
4	0*		10:00 "	5.0
5	$0.98 \cdot 10^{-6}$	↓	10:20 "	**4.5**
6	"	↓	10:40 "	4.5
7	"	↓	11:00 "	4.0

* Current on.

TABLE XXV

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 21° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		3:00 P.M.	
2	0		3:20 "	4.0
3	0		3:40 "	3.0
4	0		4:00 "	3.0
5	0*		4:20 "	3.0
6	$0.56 \cdot 10^{-6}$	↑	4:40 "	3.0
7	"	↑	5:00 "	**5.0**
8	"	↑	5:20 "	3.0
9	"	↑	5:40 "	3.0
10	"	↑	6:00 "	2.5

* Current on.

TABLE XXVI

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		4: 20 P.M.	
2	0		4: 40 "	4.0
3	0		5: 00 "	3.0
4	0		5: 20 "	4.0
5	0		5: 40 "	3.0
6	0*		6: 00 "	4.0
7	$0.7 \cdot 10^{-6}$	↑	6: 20 "	**5.0**
8	"	↑	6: 40 "	3.5
9	"	↑	7: 00 "	3.5

* Current on.

TABLE XXVII

UNINJURED SEEDLING OF *AVENA SATIVA*. TEMPERATURE 20° C.

OBSERVATION NUMBER	CURRENT STRENGTH	DIRECTION OF CURRENT	TIME OF OBSERVATION	INCREASE IN GROWTH IN 20 MIN. (IN 50 μ)
	amp.			
1	0		11: 40 A.M.	
2	0		12: 00 M.	3.0
3	0		12: 20 P.M.	3.0
4	0		12: 40 "	5.0
5	0		1: 00 "	5.0
6	0*		1: 20 "	4.5
7	$0.7 \cdot 10^{-6}$	↓	1: 40 "	**4.0**
8	"	↓	2: 00 "	4.0
9	"	↓	2: 20 "	3.0

* Current on.

A comparison of the data of the results of these experiments with the results of the previous ones shows that the same general regularity exists in both cases: a current of the order of 10^{-7} to 10^{-6} amperes caused an acceleration of growth of short duration when it passed from the base to the apex of the plant. When a current of the same strength passed in the opposite direction it either did not effect growth at all, or it caused a slight retardation of growth.

Conclusions

What are the general conclusions that may be drawn from these experiments? Do they confirm the assumption that the translocation of the growth

hormone in the coleoptile depends on electric forces and that this substance, an acid, moves through the cells of this organ primarily by means of "cataphoresis" as WENT (**12**) and KÖGL (**8**) consider it to spread? A superficial observation of the results of our experiments makes one think that they fit easily into this hypothesis. We see that a current passing from the base of the coleoptile to its apex really caused a definite acceleration of growth, and on the contrary the current passing in the opposite direction caused a decrease. But a further analysis of the same data shows that the phenomena observed by us are of a more complicated nature.

As to the acceleration of growth under the influence of the current which passed in the upward direction, it is to be noticed first of all that this acceleration lasts only for a very short time and takes place shortly after the current is switched on. Soon thereafter the growth rate decreases to the normal level and sometimes there is even a retardation in growth. Only in a few cases, where the initial growth of the coleoptile for some reason (*e.g.*, low temperature) was very slow, as in some of the experiments (tables VI–VIII), the electric current seems to have stimulated growth of this organ, increasing the rate of growth to the normal level.

The data relating to the effect of current which passed from the top of the coleoptile to its base seem to agree more closely with the WENT-KÖGL's ideas. The more lasting retardation of growth, which was observed in this case, may be explained by the immediate influence of the current upon movement of the growth hormone. But with this consideration in mind it is hard to understand the phenomenon of "aftereffect" of the current, namely, the decrease in rate of growth after the current is switched off, as seen in the results of the experiments given in tables XVII and XVIII and especially in table XIX. On the basis of the WENT-KÖGL hypothesis one would expect that after the elimination of the cause, which was retarding the normal movement of the growth hormone, its translocation in the growing zone of the coleoptile should at once increase, and this should cause a marked acceleration in growth.

Summing up our results we must say that although our experimental data do not prove the incorrectness of the view that the growth hormone has a tendency to diffuse in the plant from the negative pole to the positive one, it is difficult to coordinate them with the ideas of WENT and KÖGL. By analyzing the general features of the phenomena, which we have observed, we have a greater reason to suppose that the electric current influences the movement of the growth hormone not directly as an electrolyte, but through the complex system of the living protoplasm, upon the changeable properties of which depend both the translocation and the production of the growth substance. Our data seem to point to the complexity of the problem and suggest the need for further investigations.

Summary

1. A critical review of the recent literature on the electrophysiology of growth emphasizes a number of defects in the methods used by previous authors. The fact is stressed that polarizable electrodes must not be employed when one is working with living plants.

2. The main object of the present work was to check WENT's and KÖGL's hypothesis, which assumes that the translocation of the growth-substance in plant organs is a phenomenon of cataphoresis (**8, 12**).

3. It was found that the passage through the isolated coleoptile of oats and rye of a current of the order 10^{-7} to 10^{-6} amperes, directed from the base of the coleoptile to its apex, caused a noticeable acceleration of growth, which lasted for a short time and in most cases was followed by a definite retardation of growth.

4. If the current is increased by an initial strength during the experiment, the curve of growth rises again for a short time (tables IX, X).

5. At the lower temperatures of our experiments an electric current, which is directed to the apex of the coleoptile, sometimes does not decrease growth, but increases it somewhat, bringing growth to its normal level (tables VI–VIII).

6. A current of 10^{-7} to 10^{-6} amperes, directed from the apex of the coleoptile to its base, in most cases retards growth of the coleoptile (tables XI–XVIII, XX). This retardation continues after the current is switched off (tables XVII, XVIII).

7. Phenomena entirely analogous to the ones mentioned in paragraphs 3 and 6 of this summary were also observed in uninjured seedlings of the oats when an electric current of the order 10^{-7} to 10^{-6} amperes passed through the coleoptile or through the whole plant (tables XXII–XXVII).

8. The experiments described above do not confirm the hypothesis of WENT and KÖGL. It is difficult to harmonize their ideas with the fact that the acceleration of growth is observed only immediately after the current is on and that it lasts for a very short time. Likewise it is difficult to explain the phenomenon of aftereffect, *i.e.*, the retardation of growth after the current is switched off, by the hypothesis of these authors.

9. The results of this work seem to confirm the conclusion that the electric current affects the translocation of the hormone, not directly as an electrolyte, but indirectly through the complex system of the living protoplasm.

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ELECTRICAL POLARITY AND AUXIN TRANSPORT

W. G. CLARK

(WITH TEN FIGURES)

Introduction

The polar basal transport of the growth substances (auxins, growth hormones) in plants is a well known phenomenon, demonstrated first by WENT (**81**), and studied in detail by VAN DER WEIJ (**78, 80**). These investigators used the *Avena* (oat) coleoptile.[1] That the phenomenon is more or less general is indicated by the polar transport of auxin in roots (CHOLODNY, **19**; NAGAO **60**); in hypocotyls of *Raphanus* (VAN OVERBEEK, **62**), of *Pisum* (SKOOG, **74**); in leaves (AVERY, **2**); in the coleoptile of *Avena* (WENT, **81**; LAIBACH and KORNMANN, **40**; VAN DER WEIJ, **78, 80**; and SKOOG, **74**) and corn (VAN OVERBEEK, **63**); in *Elaeagnus* (woody cutting) (VAN DER WEIJ, **79**); in stems of *Coleus, Vicia,* and *Phaseolus;* and in hypocotyls of *Vicia, Phaseolus,* and *Lupinus* (MAI, **54**).

Other workers have reported non-polar transport of auxin in plants. HITCHCOCK and ZIMMERMAN (**31**) and ZIMMERMAN and WILCOXON (**85**) have shown an apical transport of heteroauxin (indole-3-acetic acid) and several other active compounds in stems of *Helianthus tuberosus, Nicotiana tabacum,* and in *Lycopersicum esculentum,* as indicated by induction of adventitious roots and by epinastic response of leaves. LOEHWING and BAUGUESS (**45**) have shown that heteroauxin could be absorbed by the root system of potted seedlings of *Matthiola incana* and be transported apically to increase the stem elongation over that of the controls. Both the Boyce-Thompson workers and LOEHWING and BAUGUESS have merely shown that auxin applied in high concentrations can be carried in the transpiration stream. This, of course, will not give polar transport. Higher concentrations of auxin may have effects which are not normally encountered. For example, high concentrations applied at the base of *Pisum* cuttings induce roots there, whereas in the lower, more physiological concentrations roots may be induced at the bases, only by applying auxin at the morphological tips (WENT and THIMANN, **83**).

LAIBACH and FISCHNICH (**41**) have shown that the transport of heteroauxin was not strictly polar in leaves of *Coleus* and in cotyledons of *Cucumis sativa,* but that transport could occur apically. The apical transport was much smaller than basal transport, however. AVERY (**2**), as mentioned above, found only basal transport. AVERY determined this by diffusion of

[1] Coleoptiles are leaf-sheaths which envelop growing points and first foliage leaves of grass seedlings.

the auxin occurring naturally in the leaves, whereas LAIBACH and FISCHNICH applied heteroauxin in a concentration of 0.5 per cent. in lanolin, an altogether unphysiological concentration.

JOST and REISZ (**34**) demonstrated apical transport of high concentrations of heteroauxin in *Avena* coleoptiles. This was seen from the growth of sections with their basal ends immersed in the auxin solutions, and from actual transport experiments in which auxin was collected in agar blocks at the apical ends of sections supplied with auxin in agar at the basal ends. The apical transport was, however, much less pronounced than the normal basal transport, and hence semipolarity still exists. The concentrations of auxin used in the transport experiments was 1:200,000, the length of the sections 10 mm., and the time of transport overnight. As to its effect on growth, the apical transport may have been effected by capillarity in the hollow coleoptiles. Regardless of these findings, WENT, VAN DER WEIJ, and others always observed strictly basal transport in short sections (1 to 4 or 5 mm.) when shorter periods of time (1 to 3 hrs.) were used. Moreover, as VAN DER WEIJ (**78**) mentioned, when higher concentrations of auxin are used (1:200,000), the auxin may be transported by capillarity in films of water condensed on the surfaces of the sections. At any rate, although strict polarity is always difficult to observe when high concentrations of auxin are used, the polarity still dominates apical transport. The concentrations ordinarily used are of the order of $1:10^6$ or less (WENT and THIMANN, **83**).

As has been mentioned, CHOLODNY (**19**) and NAGAO (**60**) demonstrated a polar basal transport in roots. GORTER (**28**), DE HAAN (**30**), and others claim otherwise. These controversial statements will not be discussed at this point, since they have no bearing on the polar basal transport in *Avena*.

The mechanism of polar transport

The mechanism of this polar transport is as yet little understood. WENT (**81**) showed that the transport was always in a basal direction in the *Avena* coleoptile when physiological concentrations were used; that in his special case, the velocity of this transport was about two hundred times greater than that of ordinary diffusion (being 10 mm. per hour); and that its polarity was unaffected by gravity. VAN DER WEIJ (**78, 80**) confirmed these findings and, in addition, showed that the transport would occur against a considerable concentration gradient, suffering no appreciable change. He also found that the velocity of the transport was reversibly lowered to that of diffusion when the temperature was lowered to 0° C. At this temperature, however, the polarity of the transport persisted. Polarity, on the other hand, was reversibly abolished by ether narcosis (VAN DER WEIJ, **80**). BONNER (**6, 7**) indicated that transport was dependent

upon the presence of oxygen. With regard to the independence of transport and gravity, PFAELTZER (**64**) found that 14.5 × gravity, produced by a centrifugal field, had no effect on polar transport in the *Avena* coleoptile.

PROTOPLASMIC STREAMING AS THE MECHANISM

VAN DER WEIJ (**70**) concluded that polar transport was a "vital process," but that protoplasmic streaming had nothing to do with it, since the velocity of transport was independent of temperature down to very low values, *e.g.* 0° C., while the velocity of protoplasmic streaming depended upon temperature within wide limits, citing LAMBERS (**42**). This view is supported by the observation of SCHUMACHER (**73**) that fluorescein shows polar diffusion in the plasma of stem hairs of *Cucurbita pepo,* the rate and direction of this transport being constant, while the rate and direction of protoplasmic streaming varied. BOTTELIER (**9**) favored some correlation between protoplasmic streaming and transport, finding the *velocity* of streaming (3 cm. per hour) to be *constant* between 17° and 35° C., while the amount of protoplasm in actual rotation (streaming intensity) increased with temperature, just as transport intensity increases (VAN DER WEIJ, **78**). Furthermore the velocity of transport (1 cm. per hour) is too great to be explained by a diffusion process unless it is of the nature of the model described by VAN DEN HONERT (*cf.* below). BOTTELIER (**10**) also found that oxygen limited protoplasmic streaming, as it presumably does transport.

From this discussion it is probable that protoplasmic streaming has nothing to do with the *polarity* of transport, but may be a factor in its *velocity.*

ACTIVATED DIFFUSION AS THE MECHANISM

A possible mechanism for transport is that suggested by several workers (*cf.* BRINKMAN and SZENT-GYÖRGYI, **16**; VAN DEN HONERT, **32**; SÖLLNER, **75**), which demonstrates the transport of surface-active substances at interfaces whose interfacial tension has been lowered at one end by the addition of these substances ("spreading"). The velocity of the transport of KOH in VAN DEN HONERT'S model was in one case 68,000 times greater than that of ordinary diffusion of KOH.

MASON and MASKELL (**57**) found that the diffusion of sugar in the cortex of the cotton plant complies with the rules of concentration gradients and directional flow for diffusion, but that the velocity was between 20,000 and 40,000 times greater than the ordinary diffusion of sugar would be expected to exhibit. MASON and PHILLIS (**58**) found that oxygen was necessary for such transport in the cotton plant, and state: "It is suggested that the mechanism activating diffusion consists in some special organization in the cytoplasm, maintained by metabolic energy, whereby the resistance to solute movement is so reduced that materials diffuse in the sieve-tube at rates com-

parable with those in a gas." PHILLIS and MASON (**66**), moreover, have shown that sucrose is transported against a concentration gradient in the leaf of the cotton plant. This recalls the similar transport of auxin against a concentration gradient in the *Avena* coleoptile, but is different in that the sugar transport is not as polar. The "organization" spoken of by MASON and PHILLIS is as yet unknown, although the model of VAN DEN HONERT is suggestive.

Hence "activated diffusion" may be a factor in determining the velocity of auxin transport in plants, particularly since auxin is surface active (OKUNUKI, **61**; KÖGL, ERXLEBEN, and HAAGEN-SMIT, **39**), but it is difficult to see how such a mechanism could explain polarity.

Electrical polarity

BRAUNER (**13**) found that the underside of horizontally placed plants became electropositive to the upper side. He also found that the shaded side of illuminated seedlings developed an electropositivity with respect to the illuminated side. Later BRAUNER and BÜNNING (**15**) correlated the geo-electric effect with electrotropisms. CHOLODNY (**18**) had already developed the theory that the plant growth hormone is electrically transported in the plant, accumulating more on one side than on the other, thus causing differential growth and a tropism. DOLK (**22**) assumed that the growth hormone was an acid, and suggested that the dissociated anion would be transported to the geo- or photo-induced positive pole. For a review of this literature, see WENT (**82**).

From a survey of older literature on the subject of electrical polarity in living organisms, WENT (**82**) formulated in his "Botanische Polaritätstheorie" the idea that the dissociated anion of auxin is transported longitudinally in the plant as a result of the inherent electrical polarity of the organ in question. By this time it was known that auxin was a weak acid (KÖGL and HAAGEN-SMIT, **38**). Applying his theory to seedlings, WENT suggested that the apical end of a seedling was electronegative to the basal end, and that auxin anions were electrically and polarly transported basalward. He supported this theory with experiments demonstrating that *Impatiens* cuttings exhibited a bipolar staining in acid and basic dyes (*cf.* experimental part of this paper).

Previous indirect evidence has seemed to favor a causal relation between lateral transport of auxin and bioelectric potentials in plants (BRAUNER and BÜNNING, **15**; AMLONG, **1**; KOCH, **37**; DE HAAN, **30**).

For a general discussion of transport and of the polarity theories, the reader is referred to BOYSEN-JENSEN (**12**) and WENT and THIMANN (**83**).

In conclusion, it may be said that evidence seems to favor both protoplasmic streaming and activated diffusion as velocity components in trans-

port, and bioelectric potentials as the cause of polarity in the transport of auxin in plants.

It is the purpose of the present papers to reexamine the possibility of a linkage between electrical polarity and the polar transport of auxin in plants, the first paper being concerned with electrical polarity, and the second (see July, 1937, issue) with transport and electrical polarity.

A. Inherent electrical polarity

1. Polar dye uptake.—As mentioned in the introduction, Went (**81**), to substantiate his electrical polarity theory, demonstrated a bi-polar uptake of dyes in *Impatiens* cuttings. Negatively charged (acid) dyes penetrated most at the cut apices, and positively charged (basic) dyes at the bases of immersed cuttings. Referring to his paper, it is seen that his acid dyes included light-green, acid green, quinolin yellow, and methyl orange; while the basic dyes included safranin, methyl violet, prune pure, neutral red, thionin, and gentian violet. De Haan (**30**) investigated differential staining in geotropically bending *Vicia* roots and found that basic dyes accumulated most on the convex side, as would be expected from Brauner's (**13**) finding that this side was electropositive to the concave side. De Haan classified light-green and methyl orange as "anode-coloring" (basic) which contradicts Went's classification.

With respect to such controversies, electrophoretic experiments were performed with all of the dyes used in the experiments about to be described. The dye solutions (0.5 per cent.) were made up in distilled water at pH 6.0, and a current passed from zinc electrodes through U-tubes containing the dyes. The products of electrolysis at the electrodes were washed away by automatic siphons during the current passage. At the end of the experiments, the pH values at the electrodes were found to be unchanged. When such experiments were performed, all dyes classified as acids were found to be cathodic and all basic dyes anodic. Keller has said, however, that dye particles reverse their charges in protoplasm due to the fact that colloids adsorb the particles and impart to them the charge of the colloidal particle (Keller, **35, 36**; Gicklhorn and Keller, **27**). An exception exists, he says, when the dye is in such excess that the charge of the adsorbed dye particles neutralizes that of the colloidal particle. In this case the charge of the dye particle is not reversed. Lauer (**43**) could not confirm Keller's claim that protoplasm reverses the dye-particle charge. De Haan himself found a lack of agreement between Keller's tables and "test-object" *Hedera helix*, used by Keller in making up these tables. Owing to such uncertainties, it was assumed that the present electrophoretic experiments, conducted at pH 6.0 (which is approximately that of the cell content in plants), gave the true charge of the dyes used in the following experiments with plant cut-

tings. It was likewise assumed that the plant did not alter the sign of the charge on the particles. Electrical measurements later bore out this last assumption.

PRINGSHEIM (**67**) criticized WENT's dye-uptake experiments in that the distances penetrated (1 mm. at best) observed by WENT could not allow conclusions to be drawn. According to this criticism, DE HAAN's observations, based on the number of cells stained in cross sections of *Vicia* roots, would be even less valid. From such considerations, it became of interest to repeat WENT's experiments and to test these findings with electrical measurements. The following descriptions show that WENT's observations can be clearly duplicated.

TABLE I

BIPOLAR DYE UPTAKE IN *IMPATIENS* CUTTINGS

KEY:

0	No staining
2	Slight staining
4	Up to 0.5 mm. penetration
6	1 mm. penetration
8	1.5 mm. "
10	2.0 mm. "
14	3.0 mm. "

DYE	PENETRATION	
	APEX	BASE
Negative dyes		
Trypan blue	2.0	3.0
Light-green	7.0	0.5
Methyl blue	5.0	2.0
Methyl orange	7.0	2.5
Congo red (colloidal)	4.5	6.5
Orange-G	10.0	1.0
Average	6.0	2.6
Positive dyes		
Safranin	7.0	9.3
Methyl violet	5.0	11.0
Neutral red	3.5	11.0
Janus green	5.0	9.0
Bismark brown	4.0	9.0
Thionin	5.0	7.0
Methylene blue	3.5	12.5
Cresyl violet	4.0	8.5
Brilliant cresyl blue	6.0	11.0
Nile blue A	8.0	14.0
Average	5.1	10.2

Etiolated *Impatiens balsamina* seedlings grown in sand in the dark room at constant temperature and humidity, were prepared by cutting away the cotyledons and the parts underground. Two to three cut hypocotyls were placed in upright test tubes filled with the dye solutions in 1 per cent. sucrose. The experiments were run in the dark 15 to 24 hours. After this time the hypocotyls were removed and examined in daylight, the amounts of penetration being noted and recorded as shown in table I. The concentrations of the dyes used were from 0.1 per cent. down to 0.001 per cent. In the case of the basic dyes, the higher concentrations caused more rapid infiltration of the tissue. In the case of the acid dyes, the lower concentrations frequently showed little or no staining at the apices and bases of the hypocotyls. The results of one experiment are summarized in table I, sections showing indistinguishable staining or infiltration being discarded. Three other experiments, run at other times, showed essentially the same thing, so that the table represents a typical case, and a confirmation of WENT's experiments. The numerals are represented on the same scale as in WENT's tables for purposes of comparison.

For purposes of comparison, WENT's averages for negative dyes were: apex 2.5, base 1.1; for positive dyes, apex 2.4, base 3.7. It is noteworthy that the greatest penetration WENT obtained was, at the most, one millimeter; while as much as three millimeters penetration was observed in the present work. Presumably sucrose in the solutions maintained oxidations and kept the tissues in a more normal condition during the time they were immersed.

RAMSHORN (**68**) criticized WENT's conclusions from such dye experiments, on the ground that actual electrical measurements of *Impatiens* cuttings showed electropositivity of the cut apices with respect to the cut bases. Upon repeating such measurements, measuring the dye uptake at the same time, quite the opposite was found. The experiment below is typical:

> *Impatiens* hypocotyls were cut and placed horizontally with each cut end in a cup of the dye solution in SHIVE's solution made up to 1 per cent. in sucrose. Agar–0.1 N KCl bridges from each cup led to a 0.1 N KCl solution in a cup in which the side-arm of a Zn-saturated $ZnSO_4$ half-cell could be placed. The potential differences between apex and base were measured with the WULF string electrometer described later in this paper. Table II shows the potential differences (P.D.'s) expressed in millivolts (mv.), the polarity being expressed as the sign of the tip with respect to the base. The dye penetration is represented in millimeters, the recorded figures being the averages. Several (3 to 5) sections were used for each dye solution, hence the electrical measurements represent the average of the several cuttings in parallel circuit. The dye concentrations were 0.05 per cent. All dye-charges were rechecked electrophoretically. The cuttings varied from 6 to 10 mm. in length.

In other experiments, the P.D.'s were read frequently over a period of several hours. It was revealed that the apex is at first positive[2] with respect to the base. In two hours after setting up the experiment it is electronegative, and remains so until the tissues appear abnormal (flaccid), after which the P.D. drops toward zero. This time-relation in establishing electrical polarities will be discussed in more detail under the section on P.D. gradients.

These electrical measurements confirm the dye-uptake experiments, and thus the polarity first claimed by WENT is real; that is, the apex of the *Impatiens* hypocotyl is electronegative to the base.[3]

RAMSHORN's conflicting results may be explained by the time-relations in establishing the normal electrical polarity. It will be noticed that the apical negativity did not appear at once, but two hours or more elapsed before the tip became negative.

TABLE II

MEASURED ELECTRICAL POLARITY AND BIPOLAR DYE UPTAKE IN *IMPATIENS* HYPOCOTYLS*

DYES USED	POTENTIALS		DYE UPTAKE	
	6:00 P.M.	12 HR. LATER	APEX	BASE
	mv.	*mv.*	*mm.*	*mm.*
Negative dyes				
Light-green	+11.0 (tip +)	−14.0 (tip −)	1.5	0.0
Methyl blue	+13.0	− 5.0	trace	trace
Methyl orange	+12.0	− 7.0	7.0	5.0
Methyl orange-G	+15.0	− 5.0	1.5	0.0
Average	+12.7	− 7.7	2.4	1.2
Positive dyes				
Safranin	+ 9.0	− 7.0	trace	1.5
Methyl violet	+ 7.0	−12.0	1.0	2.0
Bismark brown	+ 3.0	− 3.5	trace	trace
Methylene blue	+ 1.0	− 1.5	0.5	1.5
Cresyl violet	+ 6.5	− 2.0	1.0	2.0
Brilliant cresyl blue	+ 9.0	− 9.0	trace	trace
Nile blue A	+ 6.0	− 9.5	1.2	2.5
Auramine	+ 9.0	− 6.0	5.0	7.0
Average	+ 6.3	− 6.3	1.1	2.1
Controls				
Shive's solution	+ 8	−12	.....	.....
Crone's solution	+ 7	−20	.....	.....

* The hypocotyls were cut and placed in the cups at 5:00 P.M. (3 per dye).

[2] In this paper the electrical polarity is expressed with respect to the external circuit.

[3] The possibility remains that the electrical polarity revealed by dye uptake and the polarity revealed by measurements are alike by coincidence.

2. MEASURED ELECTRICAL POLARITY.—

a. *Brief review of the literature.*—Many attempts have been made to correlate morphological and physiological polarity with electrical polarity in living organisms (*cf.* RAMSHORN, **68**; and WENT, **82**). CHILD's school (CHILD and HYMAN, **17**; HYMAN and BELLAMY, **33**) claimed that the regions of highest metabolic rate in hydroids (apical regions) may be electronegative to other regions: LUND (**47, 50**), and LUND and KENYON (**53**), on the other hand, claimed that electrical polarity was dependent upon oxidation-reduction potentials (*cf.* discussion). Usually parts of polar tissues (apical end of hydroid stems, onion root tips) having the highest rates of oxidations were electropositive to other regions. RAMSHORN (**68**) stated that in seedlings of several different plants, electropositivity was directly linked with growth rate, such that potential distributions paralleled growth rate distributions. BARTH (**3**) showed that in several different hydroids the electrical polarity varied, some hydroids exhibiting apical electronegativity while others showed positivity. It is difficult to make *generalities* from such conflicting statements.

In general, the cortex of a root apex is held to be normally electropositive to that of the base (LUND and KENYON, **53**; MARSH, **55, 56**; RAMSHORN, **68**). In hypocotyls and coleoptiles of seedlings, the apical cortex is said to be electropositive to the basal (RAMSHORN, **68**). In the Douglas fir, the apical cortex is as RAMSHORN claimed to be the case in seedlings, *i.e.*, electropositive with respect to basal; while the apical wood is electronegative to the basal (LUND, **48, 49, 51**). In seedlings with internodes, in general, the nodal regions are electropositive to internodal zones; and the total polarity from apex (just below cotyledons) to the base of the stem shows an electronegativity of the tip to base (REHM, **70**; CLARK, this paper.) RAMSHORN (**68**), on the other hand, claimed the opposite, *i.e.*, that the tip was electropositive to the base (*cf.* discussion).

The following descriptions concern themselves directly with the determination of the electrical polarity of the *Avena* seedling, and of a few other seedlings.[4]

b. *Methods involved.*—In determining the electric potential differences (P.D.'s) in *Avena*, various types of electrodes, contacts, and recording instruments were tried. A Dolezalek electrometer was used as the recording instrument at first, but was found to be difficult of manipulation and to have too long a period. A Compton electrometer is subject to the same criticism, although it is somewhat better than the Dolezalek instrument. Lindemann electrometers are too insensitive. A potentiometer is apt to draw some

[4] Seedlings are preferred to mature, green plants because they have been more extensively studied, are more quickly obtained, and because they can be used in the dark. Light complicates the physiological behavior.

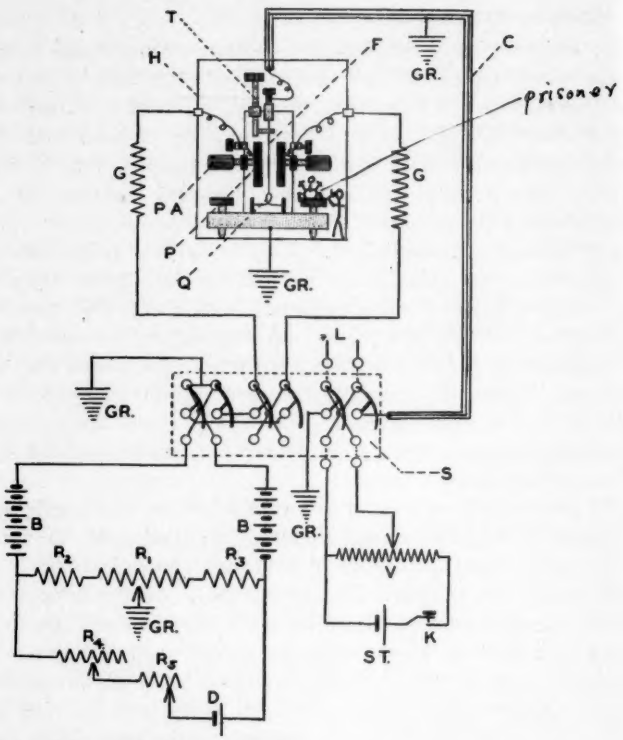

FIG. 1. String electrometer circuit. Key:

B	Six 45-volt heavy-duty Burgess B-batteries (long shelf-life)
C	Copper-tube shielding for fiber lead
D	1.5-volt dry cell battery
F	Platinum fiber (0.001 mm. diam.)
G	750,000-ohm wire-wound grid leaks
GR	Ground
H	Constant temperature housing
K	Key
L	Leads to unknown P.D.
P	Plates
P.A.	Plate adjustments
Q	Quartz fiber spring
R_1	10,000-ohm Yaxley wire-wound potentiometer
R_2, R_3	25,000-ohm Yaxley wire-wound potentiometers
R_4	750-ohm Yaxley wire-wound potentiometer
R_5	50-ohm Yaxley wire-wound potentiometer
S	Ceresin-covered mercury-in-paraffin switches
ST	Eppley standard cell
T	Tension adjustment
V	0.1–10,000-ohm plug-type Welch volt box

current from the living tissues. Hence a WULF string electrometer, constructed in the shops of this institute, was employed for the majority of the measurements (*cf.* WULF, **84**). Figure 1 represents the hook-up.

The sensitivity of the instrument in its final adjustment was more than one millimeter scale-deflection per millivolt with a scale-distance of one meter. Its period was about one second at lower sensitivities. The sensitivity depended upon the diameter of the string (platinum fiber 0.001 mm. in diameter), its tension, the distance between the plates, and the voltage across the plates. At higher sensitivity, damping increased the period to about three seconds. (This could be avoided by housing the instrument in a vacuum). The calibration curves approximated a straight line and remained constant for weeks at a time when the instrument was kept dry and at constant temperature. Because of the constant calibration and rapidity of motion of the string, readings could be made rapidly and accurately.

The distribution of potentials in the *Avena* seedling was first studied by moving contacts up and down the plant. CHAMBERS'S micromanipulators (40-pitch threading) were employed to move the electrode contacts.

Electrodes of various types were tried. It was found that bright or platinized platinum electrodes, however cleaned, gave non-reproducible readings, presumably because they were easily unpoised (*cf.* GICKLHORN, **26**; UMRATH, **77**; DORFMAN, **24**). Ag–AgCl wire loops or claws serving as contacts gave reproducible readings for a while, but demanded frequent replating. Quartz capillaries filled with fresh coagulated egg-white into which Ag–AgCl wire electrodes were set, gave reproducible readings. These were used for obtaining the internal distribution of P.D.'s. The gradients obtained with the Ag–AgCl loops and the quartz micro-electrodes, in general, gave similar results, these results being statistically comparable with those obtained with the more refined glass contacts and unpolarizable electrodes, although there was less constancy. The electrodes finally used for most of the measurements were Zn-saturated $ZnSO_4$ half-cells (*cf.* fig. 2). These remained iso-electric for months at a time. The types of contacts used in conjunction with these electrodes were varied. Usually the electrode was placed in a glass cup filled with the liquid used to make contact with the plant. A glass side-arm connected this electrode cup to the plant. At the point of contact, the side arm was fashioned into a small glass claw or loop, through which the plant led. The glass parts did not usually touch the plant, a meniscus of contact fluid performing this function. Tap water, KCl solutions, distilled water, and various nutrient solutions used in the glass contacts made no difference in the values obtained, if the same fluid was used in all contacts (*cf.* REHM, **69**). AMLONG (**1**) showed that geo-electric P.D.'s in plants depend upon the concentration of the contact fluids.

The effects of concentration were not determined in the present work, but it was assumed that the P.D's measured were not a function of the ionic species of the solution, since any of the solutions gave the same polarity, and approximately the same magnitude of P.D. (*cf.* REHM). The type of contact used for such measurements is seen in figure 2.

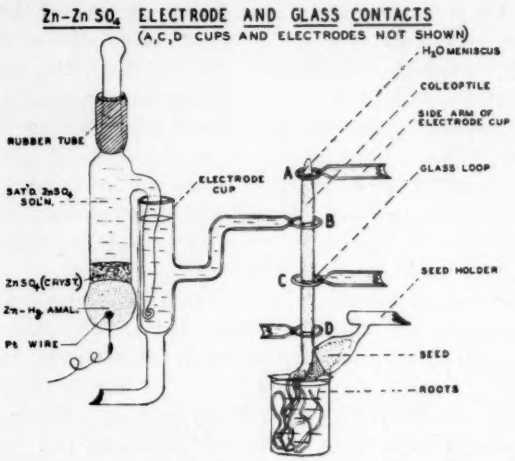

FIG. 2. Diagram of electrode and glass contacts used in measuring the P.D. of *Avena* coleoptiles.

A variation of this type of contact consisted in the use of a cotton or linen thread which was made wet by being threaded through the side arm of the electrode cup to the fluid in the cup. This thread then could either be wrapped around the part of the plant being studied, or could merely touch it (REHM, **69, 70**). Such threads maintained an isoelectric condition satisfactorily if they were occasionally washed.

The most useful type of contact, shown in figure 3, consisted in the following: Long, thin agar threads (made up of 2 per cent. agar in tap water, distilled water, 0.1 N KCl, SHIVE'S, CRONE'S, or HOAGLAND'S solution), 0.5 mm. or less in diameter, were made to hang from paraffined glass capillaries filled with the same agar. This was done by pushing an agar-filled capillary into more of the same agar, thus partly displacing the agar in the capillary as a thread. These capillaries were set in larger paraffined glass tubes filled with the same agar, which in turn were mounted in upright rows in a moist chamber. The glass tubes filled with agar led outside the chamber to paraffined electrode cups filled with the solution of which the agar was made. Zn–$ZnSO_4$ electrodes were placed in these cups. The agar threads hanging from the capillaries made contact to seedlings in the moist chamber,

being held to the plant by a drop of 15 per cent. gelatin. Several such fixed contacts could be made to each plant; and several plants could be set up simultaneously. The threads were prevented from drying by maintaining the chamber at near saturation with water vapor from strips of moist filter paper on the sides of the chamber. This method has the advantage that the agar threads remain fixed to the plant in the same position, being carried by upward growth without stimulating the plant. Seedlings, to which several glass contacts were fixed, frequently grew up through the more apical contacts, necessitating moving the apical contact back up to the tip. Such manipulations, however carefully done, usually stimulated the plant, thereby altering the P.D.'s.

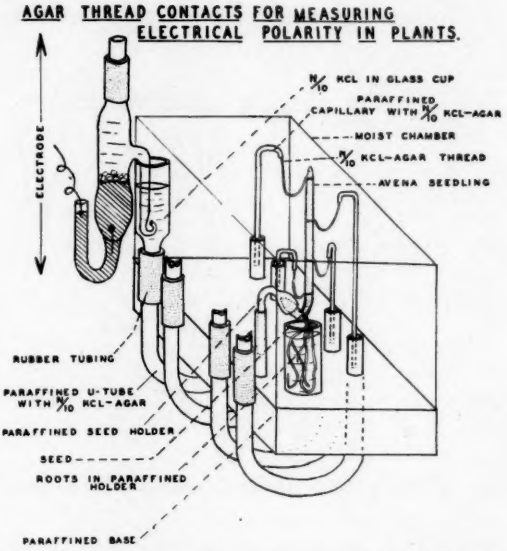

FIG. 3. Diagram of agar-thread contacts for measuring electrical polarity. Note that only one of several plants is shown, and only one of four electrodes is shown in the drawing.

c. *Electrical polarity and P.D. distributions in the Avena coleoptile.*— As described in an earlier paper (CLARK, **20**), the P.D.'s in the *Avena* seedling, obtained by manipulation of two contacts up and down the plant, were not constant over any considerable period of time, since the manipulation always resulted in changes of P.D.'s. Such "handling reactions" are illustrated in figure 4. This was usually true regardless of the care taken in making the manipulation, even if contact to the plant was made merely by a meniscus of water from the contacts. Moreover the orange light in the

dark room proved to be a stimulus.[5] Plants left in complete darkness gave variable P.D.'s as soon as this light was again turned on. Again, if two contacts were left on the plant in a fixed position, one at the tip and the other at the base of the coleoptile, and the plant left in complete darkness, con-

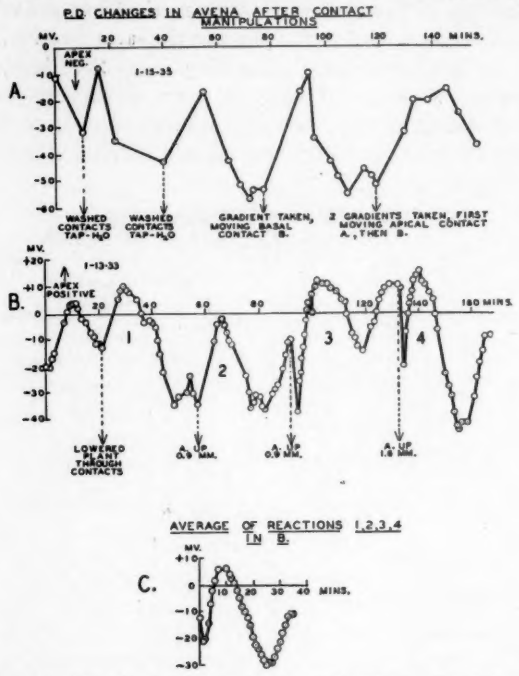

FIG. 4. Changes in P.D. of *Avena* coleoptiles produced by manipulation of contacts.

stancy of the P.D. was established only after an average time of 110 minutes. This constancy was abolished occasionally by the appearance of rhythmical P.D. changes, due probably to growth of the coleoptile up through the contacts, and also due to nutations which cause rubbing against the glass of the contacts. Clean glass adheres rather strongly to the *Avena* coleoptile cuticle, and considerable stimulation is caused by such movements. This rhythmical effect was avoided by the use of glass contacts dipped into 15 per cent. gelatin, thereby rendering the contacts slippery to the cuticle, or by recourse to the agar thread technique described above.[6]

[5] The light used to illuminate the dark room was filtered through a Corning filter no. 348, which cut out all wave-lengths below 575 mμ. No phototropism occurred in this light.

[6] Stimulation by contact is discussed by PFEFFER (65).

To obtain the normal P.D. distribution in the coleoptile, therefore, four fixed contacts were made to the plant (*cf.* fig. 2, A, B, C, D, and fig. 3). The plants were left in complete darkness. After constancy of P.D.'s obtained (80–120 minutes), the P.D. distribution could be easily and quickly determined. Figure 5 A represents the relative constancy of the P.D. between tip and base of *Avena* coleoptiles with fixed, gelatin-dipped glass electrodes; while figure 5 B represents the same type of experiment in which, however, the contacts were not gelatin-dipped.

Figure 5 C shows the much greater constancy obtained when the agar-thread contact method was used. The constancy lasts several hours. The figure has fewer points than either 5 A or B, but that no changes occur between the points has been verified by many other determinations.

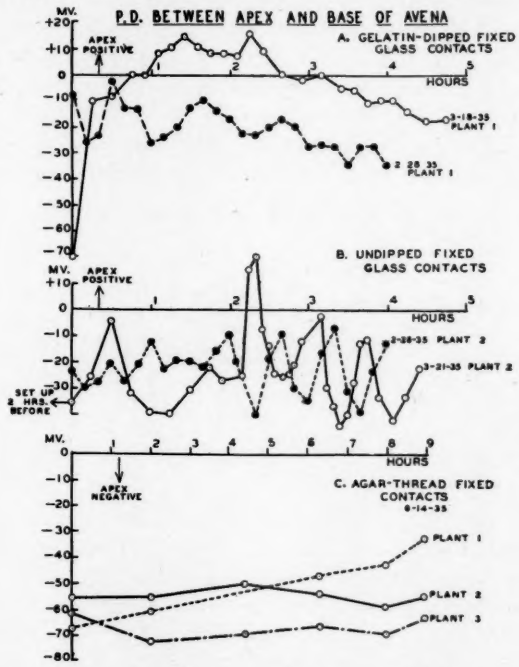

FIG. 5. P.D. between apex and base of *Avena*.

A. P.D. between apex and base of the *Avena* coleoptile, on using glass contacts dipped in gelatin.

B. P.D. between apex and base of *Avena* coleoptile, on using contacts not gelatin dipped.

C. Establishment of constant P.D. between apex and base of *Avena* coleoptile using agar-string contacts.

Figure 6 A illustrates the electrical polarity and P.D. distribution in the *Avena* coleoptile of intact plants as determined with the four fixed, gelatin-dipped glass contacts, the plant being in total darkness, and constancy having been obtained. The electrode cups each contained a Zn–$ZnSO_4$ electrode, each of which was isoelectric to all the others both before and after each experiment. The P.D. between each contact position to the plant was obtained by manipulation of mercury-in-paraffin switches outside the experimental chamber in which the plants were housed. Figure 6 B illustrates a similar gradient taken with only two glass contacts, the basal contact being fixed, and the apical contact being moved toward the base by means of the micro-manipulators. Every few mm., a reading was taken. After the contacts touched each other, the apical contact was again moved upward, readings being taken every 5 mm. The movements of such fluid contacts up and down the coleoptile did not wet the surface and thus invite electrical shunting (*cf.* ROSENE, **71**), because of the fatty nature of the cuticle. It will be noticed that the gradient taken by the manipulation down the coleoptile differs from that taken on moving the contact back up the coleoptile. After such manipulations it is found that the P.D.'s vary considerably, and sometimes the polarity is reversed for considerable time (*cf.* figure 4). Figure

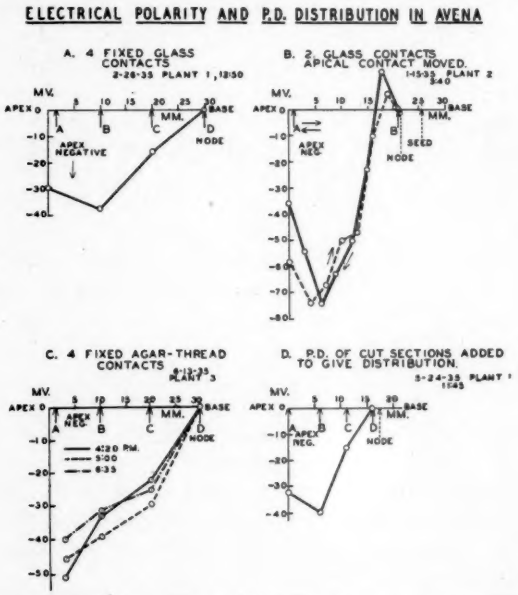

FIG. 6. Electrical polarity and P.D. distribution in the *Avena* coleoptile with various contacts and arrangements.

6 C represents a similar gradient, the contacts in this case being four fixed agar threads as described above. The three curves in C represent three gradients taken on the same plant at different times, showing that the P.D. distribution remained constant for a considerable time. Figure 6 D represents the P.D. distribution calculated from the individual P.D.'s of cut-sections of a coleoptile (*cf.* section on P.D.'s of cut sections). In all of these curves, electronegativity of the tip is represented on the ordinates, and the length of the coleoptile is represented on the abscissae, A, B, C, and D representing points of contact to the coleoptile from tip to base. The potential at D is taken as the reference zero. The P.D. from A to D is always equal to the sum AB + BC + CD.

From the above experiments it is clear that the tip of the *Avena* coleoptile is normally electronegative to the base.

d. *Electrical polarity and P.D. distribution in Pisum, Impatiens, and Zea.*—*Pisum sativum* seedlings were studied for the normal electrical polarity and P.D. distribution. Contacts were made to etiolated plants with the linen threads previously described, the threads being wrapped around the zone to be measured. There were several electrode contacts to each plant, represented by the positions on the abscissae corresponding to the points plotted in figure 7 A, 1, 2, 3. The P.D. obtained between each contact was found to be fairly constant for several hours at a time. The measurements were made in the darkroom in weak red light. Figure 7 A represents typical gradients. It will be noticed that nodal zones are electropositive to the internodal zones, and that there exists an electronegativity of the tip with respect to more basal regions. This confirms REHM'S (**70**) findings on *Phaseolus,* and disagrees with RAMSHORN'S (**68**) finding that the tips of *Asparagus* seedlings are electropositive to the basal regions, although the nodal zones were positive to internodal zones.

Etiolated *Impatiens balsamina* seedlings were set up in the same way as *Pisum* seedlings were, and the P.D. distribution recorded. The hypocotyls are without nodes, and the plants were only seven centimeters in height. Figure 7 B represents the distribution found.

Zea mays seedlings 5 to 7 cm. in height were set up in the same way as has been described for the *Avena* seedlings, four fixed contacts being made by means of agar threads. The polarity and P.D. distributions found corresponded very closely with those recorded for *Avena.*

It is concluded from these observations that in *Pisum, Impatiens,* and *Zea,* the tip of the etiolated seedling is normally electronegative to the basal regions. This shows that the electrical polarity of the *Avena* coleoptile is not unique in its apical negativity.

e. *Internal electrical polarity in the Avena coleoptile.*—The above discussion concerns the electrical polarity and P.D. distribution measured on

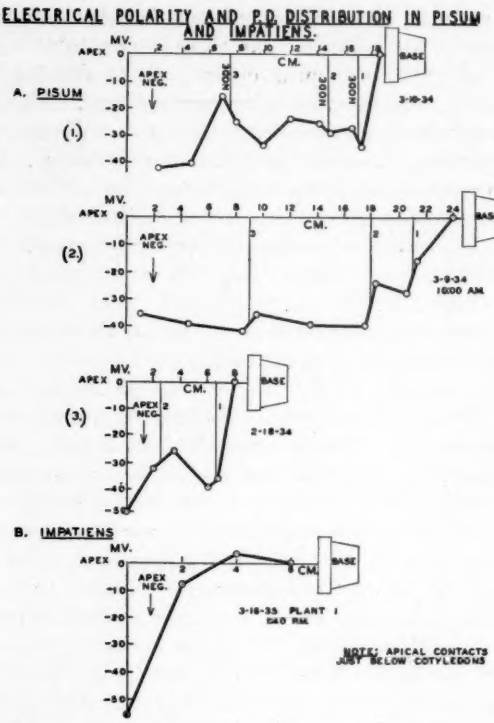

FIG. 7. Electrical polarity and P.D. distribution in *Pisum sativum* and *Impatiens balsamina.*

the cuticle, thus the *external* polarity. It is conceivable that the distribution of *internal* P.D.'s might be different, as suggested by LUND's (**49, 51**) findings in the Douglas fir. Here the apical wood was electronegative to basal wood, while the apical cortex was electropositive to basal. For this reason a few experiments were performed on the *Avena* coleoptile, in which the P.D. distribution beneath the cuticle was examined. Quartz microelectrodes (*cf.* descriptions in this paper) were inserted in coleoptiles by means of micromanipulators, and the electrical polarity was measured. The electrodes are illustrated in figure 8. They maintained an isoelectric condition very satisfactorily for two or three hours. Figure 8 A shows the P.D. between two such electrodes inserted in a coleoptile. One electrode was inserted in the wall of the coleoptile at the apex, and one at the base, both electrodes being in the same side-wall, but being inserted from opposite sides of the coleoptile. Traumatropisms toward the sites of insertion occurred

after about 30 minutes, as diagrammed in figure 8 A. Immediately after insertion, the apex of the coleoptile became electropositive to the base, but after 20 or 30 minutes, as seen in figure 8 A, the tip became negative. It was considered impracticable to insert several such electrodes, or to reinsert the same two electrodes at several different loci on the coleoptile in order to obtain the P.D. distribution. This would have resulted in even more P.D. variation than is depicted in the figure; hence, in order to obtain the distribution of internal P.D.'s, a different technique was employed. Several longitudinal slits were made down the coleoptile using a sharp razor. Each slit had its counterpart on the opposite side of the coleoptile in order to compensate the wounding effects. Such coleoptiles will remain straight, whereas the ones in which the microelectrodes were inserted showed traumatropisms. Glass-loop contacts, such as were described above, made contact with the plant, one at the tip and one at the base of the coleoptile. The basal contact was racked up the coleoptile toward the apex by means of the micromanipulator. When a contact was centered over a slit, presumably the potential internal to the cuticle was measured. Figure 8 C shows the distributions obtained in this way. The vertical lines, a, b, c, d, and e represent the loci of the slits in the coleoptile. Curve I represents the distribution obtained by racking the basal contact up to the apex, whereas curve 2 represents the distribution obtained by racking it back down a few minutes later. Curve 3 represents the distribution obtained by racking it back up 20 minutes later. Figure 8 B illustrates the changes in P.D. between the apex and base of the coleoptile before and after each distribution was determined. It will be noticed in curve 2 of figure 8 C, that the readings were taken with the contacts centered on the slits. The distribution is very similar to that obtained on the intact cuticle. Curve 1 shows the same thing, with the exception that one contact was centered *between* two slits, thus on the intact cuticle (between d and e). Curve 3 shows the type of curve obtained when no attention was paid to the position of contacts with respect to slits, *i.e.*, the distribution was taken at more frequent loci, regardless of the slit positions. The general electrical polarity is the same as that on the intact cuticle, but the curves are not smooth. This indicates a *radial* P.D. between cuticle and the internal tissues. The curves are smooth if contacts and slits coincide.

The following section (f) will show that cut sections are of the same electrical polarity as has just been described; and, as seen in figure 6, the sum of the section P.D.'s of a coleoptile give a P.D. distribution for the coleoptile which is comparable to that in an intact plant.

The evidence presented indicates that the distribution of internal P.D.'s in the *Avena* coleoptile is the same as that on the outside (cuticle). The polarity is the same, *i.e.*, the tip is electronegative to the base.

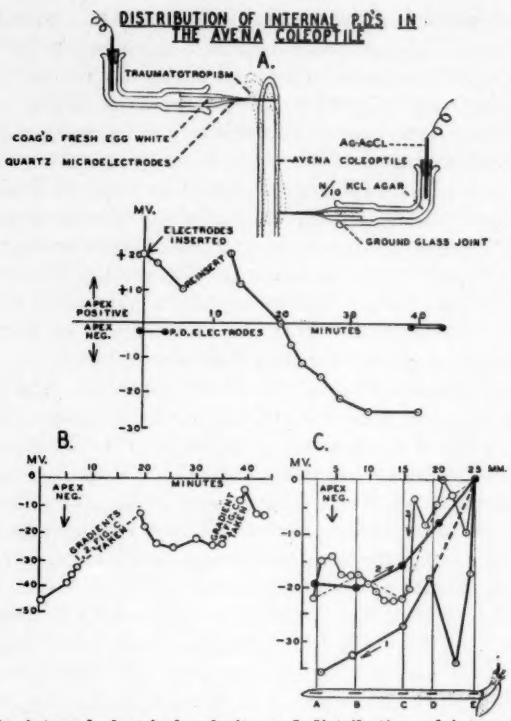

FIG. 8. The internal electrical polarity and distribution of internal P.D.'s in the *Avena* coleoptile.

f. *Electrical polarity of cut sections.*—The P.D.'s and electrical polarity of cut sections of the *Avena* coleoptile were then determined in several different ways. One method involved cutting the sections with two parallel razor blades separated by a brass strip. The cut surfaces were washed by placing the sections upright on wet filter paper for an hour. The sections were then carefully transferred to the experimental chamber, using eye-forceps. Contact was made to individual sections with the agar-thread method, or with agar strips. 0.1 N KCl agar was used in most cases. The strips or threads led through paraffined glass tubes to electrode cups outside the chamber. $Zn–ZnSO_4$ electrodes were placed in these cups, and the P.D.'s measured. The chamber was maintained at a high vapor pressure by means of strips of moist filter paper.

Several hundred measurements on 3-mm. sections revealed that the cut apical surface was always electronegative to the cut basal surface from 1 to 15 mv., the magnitudes depending upon the time at which the measurements were made.

Another method involved placing from 12 to 20 sections on an agar block, and placing a similar agar block on the tops of the sections. 0.1 N KCl agar strips made contact with these blocks and to the cups outside the chamber. Here, therefore, the average P.D. of several sections in parallel was measured. The same result was obtained, *i.e.*, apical negativity. This latter method will be discussed again in a later paper.

A third method involved making contact with several places on longer sections with agar threads held in place with a drop of gelatin, as described in an earlier section of this paper. The cut surfaces were usually electronegative to the intact cuticle (another indication of a "radial" polarity), but the apical cut surface was always negative to the basal cut surface.

Using the method by which 20 sections were measured in parallel at the same time, the relation of the length of the section to the P.D. of the section was determined. It was usually found that time was required before the maximum P.D. was established (Presumably this was due to the diffusion of ions from the sections into the agar blocks). For this reason, the P.D.'s were allowed to reach their maximum values before plotting against length. This time function was more pronounced in longer sections. Figure 9

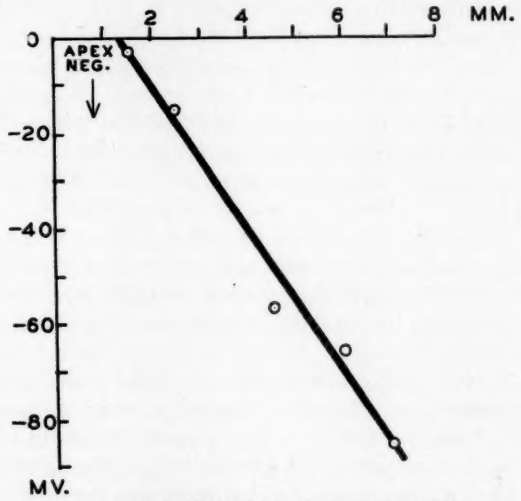

FIG. 9. The relation of section length to P.D.

shows the length of the section in millimeters plotted against P.D. in millivolts, after this maximum P.D. had been reached in all sections (5 hours).

In the section on P.D. distribution in intact plants (fig. 6), it was seen that the sum of the several P.D.'s along a coleoptile was equal to that measured from apical contact to basal contact. In figure 9 it is seen that

this principle of summation again holds, since the magnitude of the P.D. of cut sections is directly proportional to the length of the section. (*Cf.* LUND, **47, 51,** and ROSENE, **71**, on the principle of summation of P.D.'s.)

Cut sections of *Vicia faba* and of *Pisum sativum* showed the same electrical polarity as *Avena* coleoptile sections. In the section on dye-uptake, it was seen that *Impatiens* cuttings also showed apical negativity. Thus the phenomenon seems quite general. In *Vicia* and *Pisum*, the P.D. magnitudes varied from a few millivolts to 30 or 40 millivolts, depending upon the length of the section.

g. *Geoelectric effect.*—During the measurements of section P.D.'s, it was noticed that inverted sections exhibited an inverted electrical polarity. A section seemed to show negativity of the end oriented upward regardless of whether this end was morphological tip or base.

The experimental procedure usually consisted in inverting single sections and measuring the individual P.D.'s of these sections, or by placing 12 to 20 sections on one agar block, making contact with this block and with the other cut surface with a similar agar block, thus obtaining the average P.D.'s of the lot of sections in parallel. Measurements were made immediately, when possible, upon inverting the sections. The polarity of the inverted sections showed an immediate inversion of electrical polarity. The time relations of the inversion have not been carefully studied, but the establishment of the inverted polarity seemed to take less time than the geoelectric effects of BRAUNER (**13**). It was noticed, however, that this geoelectric effect was not maintained indefinitely, particularly in the shorter sections. The original polarity (apical negativity) returned within 60 to 120 minutes, depending upon the length of the sections. Figure 10 A shows the course of the P.D.'s of inverted sections during a period of time. The sections were cut at 9:30 A.M. and placed on wet filter paper in an inverted position. The experiment was set up at 11:30 A.M., the first readings being taken at 11:40 A.M. During this time, the inverted polarity had attained a considerable magnitude.

Figure 10 A, curve 1, shows the change in P.D. of control upright 3-mm. sections. They exhibit normal apical negativity. Curve 2 is for inverted sections 3 mm. in length; curve 3, 5 mm.; curve 4, 7 mm. in length. It is seen that the longer the sections, the greater the magnitude of the inverted polarity; and that the greater the magnitude of this inverted polarity, the longer the time necessary to reestablish the normal polarity. The abscissae are in hours after cutting the sections. Figure 10 B represents data from the same experiment. The P.D.'s of sections with inverted polarity are plotted against the length of the sections in millimeters. The P.D. values used are those of maximum magnitude, *i.e.*, at the first measurements, as represented on the zero ordinate of figure 10 A. It is seen that a nearly

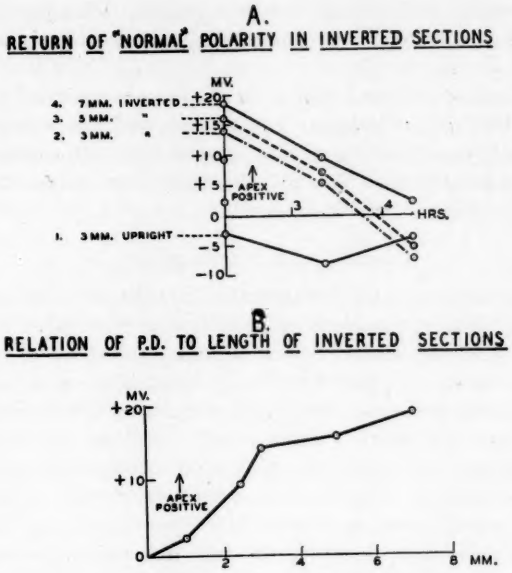

FIG. 10. "Geoelectric" polarity of sections.

direct proportionality exists between length and the P.D.'s of inverted polarity, thus indicating a similarity to upright sections which exhibit the same direct proportionality.

The possibility remains that the normal polarity of upright sections of plants is partly a result of the position of the plant or section with respect to gravity, *i.e.*, that the electrical polarity *is*, partly, a geoelectric effect. This scheme is complicated by the return to normal polarity with time. It is not known what the effect of inverting intact plants, *e.g.*, roots or shoots, has on their electrical polarity. This problem is being investigated. The establishment of positivity of the under side of inverted sections recalls to mind the similar establishment of positivity of the under side of horizontally-placed plants (BRAUNER **13**).

That the geoelectric polarity or inverted polarity is not a phenomenon confined to *Avena* sections was shown by the fact that 5-mm. inverted *Pisum* sections developed the inverted polarity. In this case, however, more time was required to establish the inversion. On first inverting, the polarity was found to be inverted a few millivolts, the maximum inverted polarity becoming established only after 3 or 4 hours. Since BRAUNER (**14**) showed that the geoelectric effect varied in the seed coat of various plants, depending upon the membrane structure, this is not surprising. It is likely that other

plant sections would exhibit their own peculiarities. This individuality was also observed by BRAUNER (**13**) for geoelectric effects in horizontally placed plants.

From the section f on electrical polarity, it was concluded that the normal, inherent electrical polarity of intact plants and cut sections of *Avena*, *Zea*, *Pisum*, and *Impatiens* was an electronegativity of the apical parts with respect to more basal parts. This polarity exists internally as well as externally, and is not directly related to growth.

Discussion and conclusions

It has been experimentally demonstrated that the normal electrical polarity of several seedlings is an electronegativity of tip to base. Several views are held as to the mechanism of the origin of this electrical polarity.

LUND (**47**) has claimed that electrical polarity is the result of oxidation-reduction potentials such that usually, but not necessarily, regions of highest rates of oxidation are electropositive to other regions (or, in the thermodynamic sense of red-ox potentials, the ratios of oxidant to reductant are different in the different ends of the structure concerned). A detailed discussion of the theory is out of place at this time (LUND, **50**), but certainly the theory is thrown into doubt by the fact that red-ox potentials can be measured only by indifferent electrodes, and not by non-polarizable electrodes such as used by LUND. FRANCIS (**25**), BEUTNER AND LOZNER (**5**), RAMSHORN (**68**), STERN (**76**), and DORFMAN (**23, 24**), have all criticized LUND'S theory on this basis. STERN (**76**) and MARSH (**56**) stated that if the living membranes, to which LUND made contact with non-polarizable electrodes, acted as indifferent, metallic conducting electrodes, the P.D. measured could be the same as LUND'S hypothetical oxidation-reduction chain interposed between the contacts. While it is improbable that these membranes act as metallic conductors, yet the possibility remains that they may do so.[7]

Bioelectric potentials, as linked with oxidative processes, can be explained by other mechanisms as well as by oxidation-reduction potentials (STERN, **76**; DORFMAN, **23, 24**; BEUTNER AND LOZNER, **5**; FRANCIS, **25**). Most of these explanations are based on the effects of oxidations on diffusion potentials or membrane potentials.

The oxidation-reduction polarity theory, moreover, is thrown into doubt by the experiments of DORFMAN (**23, 24**), who showed that the oxidation-reduction polarity of the frog's egg was opposite in sign to the bioelectrical polarity measured with non-polarizable electrodes.

[7] SCHOTT and BORSOOK (**72**) have shown the possibility of metallic electron conduction between enzyme centers in *E. coli*. FETCHER (dissertation, University of Chicago, 1934) demonstrated the possibility of electron conduction in membranes which are composed of conductors of the second class (LILLIE, 44).

The bioelectrical polarity of the *Avena* coleoptile is certainly not directly linked with respiration in the different regions of the coleoptile, since BONNER (**6**) showed that there was no distribution of respiration in this organ. Moreover, from data to be published shortly, reduced ascorbic acid (vitamin C) is found in highest concentration in the apex of the *Avena* coleoptile, the concentration decreasing basally (cystine, cystein, and glutathione are not present). It was also indicated that the reverse relation held for the distribution of oxidized ascorbic acid. This does not conform with LUND'S oxidation-reduction polarity (LUND, **50**), since by this theory, the tip would ' ually be electropositive. This is assuming, however, that the oxidation-reduction potentials of ascorbic acid could play a part in the electrical polarity. (For exceptions to this polarity rule, see LUND, **50**).

With regard to the disagreement between the findings presented in this paper and those described by RAMSHORN (**68**), the following discussion becomes pertinent: RAMSHORN made measurements of the electrical polarity of several different seedlings and roots, and showed a parallelism between growth and electropositivity. Regions of highest growth-rate were electropositive to other regions. Temperature changed both in the same way, and applied potentials accelerated growth if the applied polarity coincided with the measured inherent polarity; and, conversely, inhibited growth if the polarities were opposed. On page 741 of his paper, RAMSHORN presents a series of curves of the gradient of electric potentials from tip to base in *Helianthus* hypocotyls after stimulation by shaking. After stimulation, the tip became electronegative to the base presumably within a few seconds. In 15 minutes the tip became electropositive, and in 75 minutes the magnitude of this positivity had diminished only a few millivolts. This roughly confirms LUND'S (**52**) finding that the electropositivity of the apex of the Douglas fir decreases or that the tip even becomes negative on mechanical stimulation.

In the present study of the *Avena* coleoptile, reliable constancy of P.D.'s was not obtained until 90 to 110 minutes after setting up the experiment, as much care as possible being taken not to stimulate the plants during this operation. Moreover, constancy was not good unless the plants were in complete darkness; and manipulation of contacts from point to point involved considerable stimulation.

RAMSHORN'S correlation between growth and electrical polarity might, in my opinion, suffer a reversal in some cases, particularly in *Avena*, if the time relations, contact manipulation, and light conditions of his experiments were reinvestigated, especially with regard to the constancy of observed P.D.'s over longer periods of time.

BARTH (**3, 4**) observed that apical positivity and organic polarity in the hydroids are not correlated, but that either apical or basal positivity

may be correlated with organic polarity, depending on the hydroid used. This lends no support to RAMSHORN's positivity theory; nor do REHM's (**70**) measurements on *Phaseolus*, in which he found the tip of the plant electronegative to the basal regions.

CZAJA (**21**) says that the auxin-transport itself electrically polarizes the plant, thus roughly supporting RAMSHORN's statement that auxin changes the P.D.'s and growth rate. CZAJA, however, is largely theoretical in his consideration, and bases his assumptions on results obtained with unphysiological concentrations of auxin. In the light of unpublished experiments of my own, the effect of auxin on plant potentials is a real one, but possibly one not closely linked with the normal inherent polarity. DE HAAN (**30**) is also of this opinion.

Experiments on the effect of gravity on the *Avena* coleoptile P.D.'s have revealed that the electrical polarity can be changed or inverted by inverting their morphological axes. This polarity inversion is not permanent, the original polarity returning. Hence "normal" electrical polarity is not exclusively and directly caused by geoelectric potentials; but it is possible that they are contributing factors. This possibility is being examined.

The mechanism of the origin of electrical polarity might be linked with the phenomenon of polar conductance observed, *e.g.*, by BRAUNER (**14**), METZNER (**59**), and GUHA (**29**). Plant tissues have been shown to exhibit a selective ionic permeability so that an electric current is conducted more easily in one direction than in the other. Investigations are under way to see if there is any relation between polar conductance, electrical polarity, and polar transport of auxin.

Summary

1. Theories of the cause of polar transport of auxin in plants are discussed. The electrical theory has been accepted by many workers as one of the most plausible (*cf.* second paper, following issue of PLANT PHYSIOLOGY).

2. WENT's bipolar dye-uptake experiments on *Impatiens* cuttings, used to substantiate his electrical transport theory, are repeated and confirmed. Positively charged dyes are taken up most by bases, negatively charged dyes by apices, of *Impatiens* cuttings. This is in agreement with the fact that electrical measurements show that *Impatiens* cuttings have apical electronegativity.

3. Intact *Avena* and *Zea* coleoptiles, *Pisum* stems, and *Impatiens* hypocotyls exhibit apical negativity when constancy of P.D. measurements is obtained. Various methods of measuring this polarity are discussed.

4. Cut sections of *Avena* and *Zea* coleoptiles, and of *Pisum* and *Vicia* stems exhibit the same polarity found in *Impatiens* cuttings, *i.e.*, apical negativity. Time is required to establish this polarity. The P.D.'s of sections are directly proportional to the length of the sections.

5. The internal electrical polarity of the *Avena* coleoptile is the same as the external.

6. Inverting sections inverts their electrical polarity, *i.e.*, the morphological apices become electropositive to the bases. This inverted polarity disappears with time. It is proportional to the length of the sections as in the case of upright sections; and the time of disappearance of the inverted polarity is proportional to the length of the sections.

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EFFECT OF ETHYLENE AND CERTAIN METABOLIC GASES UPON RESPIRATION AND RIPENING OF PEARS BEFORE AND AFTER COLD STORAGE

ELMER HANSEN AND HENRY HARTMAN

(WITH FIVE FIGURES)

Introduction

Previous work by the writers (**6**) has shown that the ripening rate of newly picked pears can be markedly stimulated by ethylene or gases of similar properties naturally evolved, but fruit treated after being held for prolonged periods of cold storage is not similarly affected. As a tentative explanation of these differences, it was suggested that the effects of ethylene are confined to a pre-ripening period, and later ripening changes apparently are unaffected by the presence of the gas in the atmosphere surrounding the fruit. These observations are in agreement with those previously reported by KIDD and WEST (**1**) for apples.

The present investigation was undertaken to further study the effects of naturally occurring ethylene upon the ripening processes in pears and to more definitely determine if there is a stage in the life of the fruit when treatment with this gas is most effective.

Material and Methods

The varieties of fruits used in these experiments were Bartlett, Bosc, Comice, and Anjou pears. With the exception of the first mentioned variety, these were grown in the Medford (Oregon) district. Fruit intended for treatment immediately after picking was gathered at approximately ten-day intervals, beginning a month or more prior to and extending considerably beyond the regular commercial harvest season. Fruit intended for storage studies was picked at one date when the proper picking stage for the variety had been reached.

Immediately after picking or removal from storage, a pressure test was taken of each lot, which was then subdivided into two duplicate lots of 10 to 15 pears each. These were weighed, placed in five-gallon glass jars provided with air-tight lids, fitted with inlet and outlet tubes.

To prevent any accumulation of gases that might be effective in stimulating ripening of fruit within the containers, the untreated lots were provided with constant aeration in the following manner: An air line with an inlet to a fresh air supply was installed in the ripening room. The jars containing the untreated samples were connected to outlets on the air line and continuous ventilation was provided by means of a vacuum pump operating

on the reverse end. The incoming air before passing over the fruit was brought to room temperature and conditioned for humidity by passing through a five-foot length of pipe and finally bubbling through one-half inch of water maintained at room temperature.

The treated fruit was supported in the jars on a false bottom, permitting the use of a 15 per cent. KOH solution beneath to absorb the carbon dioxide evolved and to maintain a humidity comparable to that in the ventilated jars. Atmospheric concentration of oxygen was maintained by the addition of pure oxygen from a constant water level siphon. In those lots treated with ethylene artificially applied, the concentration of gas used, in all cases, was one part by volume to 1000 parts of air. In the tests with naturally evolved gases, three or four ripe pears were enclosed in the jar together with the fruit to be ripened. The fruit was kept in the containers continuously during the time of treatment except for a period of one to two hours daily when removed to determine the rate of respiration. In the cases where ethylene was applied, the gas was replenished after each removal of the fruit from the jars. The KOH solutions were renewed often enough to assure efficient absorption of carbon dioxide.

Respiration determinations were conducted in a room maintained at a temperature of 65° F. Although thermograph records showed in some cases a variation of two to three degrees, this was not considered to have materially affected the differences in rate of respiration observed between treated and untreated lots, since both were always subject to the same temperature changes. The method of HARDING, MANEY, and PLAGGE (7), with slight modifications, was used for carbon dioxide determinations. The carbon dioxide was absorbed in 0.15 N barium hydroxide and titrated directly with the N/10 hydrochloric acid, using thymolphthalein as indicator. Determinations were made daily for a period of seven to nine days with the storage series, but with newly-picked fruit this period was considerably extended because of the longer period of time required for ripening. At the end of the experiment, loss in weight for each lot was determined.

The rate of ripening of treated and untreated lots was determined by the comparative softness of the flesh as indicated by the Oregon pressure tester, and by the number of days required for the fruit to attain an edible condition. With the newly picked fruit, where carbon dioxide determinations were extended in some cases over a period of thirty days or more, the number of days required for ripening only was used as an index of ripening rate. With the storage series, a pressure test was made on one-half of both treated and untreated lots at the end of nine days and the remainder kept to determine the number of days required for ripening. If the fruit was ripe before the expiration of nine days, the entire sample was used for a pressure test.

Experimental results

I. Effect of naturally evolved gases and of ethylene artificially applied upon respiration and ripening of newly- picked fruit

Selection of samples and treatment.—The samples of each variety used in the experiments with newly-picked fruit were selected from single trees and represented the average size of the fruit at the time of gathering. The first two pickings of Bartlett were made approximately two months prior to the commercial harvest season, but because of the small size and undeveloped condition of these fruits no treated lots were included. The data are presented, however, to show the trend in respiration at this early period in the season. A sample of the third picking was treated with ethylene gas, since no ripe pears were available at that time, but all subsequent lots of newly-picked fruit to be treated were inclosed with ripe pears as indicated in the general methods.

TABLE I

Effect of ripe fruit upon the ripening of newly-picked Bartlett, Anjou, and Comice pears

Variety	Date picked	Days required for ripening		Difference in days required for ripening
		Treated	Untreated	
Bartlett	7–22*	8	20	12
	8–20	8	14	6
	9–4	7	12	5
	9–17	6	8	2
Comice	8–16	20	†	.....
	8–30	15	†	.....
	9–20	11	30	19
	10–8	8	15	7
Anjou	8–16	18	†	.....
	8–30	12	†	.....
	9–16	12	35	23
	10–8	8	15	7

* Treated with ethylene.
† Failed to ripen in 30 days.

Effect upon rate of ripening

That the gas produced by ripe pears does have a very pronounced effect upon the ripening of newly-picked fruit is evident from these studies. As shown in table I, the treated lots, subjected to the influence of several ripe pears in closed containers, ripened in comparatively short periods of time.

Duplicate lots, however, that were not subjected to the influence of ripe fruit, but kept under constant ventilation with fresh air, either failed to ripen or were markedly delayed beyond the time required for ripening of the treated fruit.

But it is clearly evident from the results obtained that while the period required for ripening can be significantly reduced by these naturally occurring emanations, the magnitude of the decrease obtained depends upon the degree of maturity of the fruit at the time of picking. Thus, with an early picking of immature Bartlett, there was a difference of 12 days between the ripening time of treated and untreated lots. With post-mature fruit, however, the ripening period of the treated lot was only two days less than that of the untreated. Similar results were observed with Anjou and Comice pears. These varieties respond to ethylene over a longer period, however, than do Bartlett. Reference to table I shows that while both of these varieties were markedly affected in rate of ripening by the emanations from ripe pears, the greatest response was obtained in those lots picked early in the season. Thus, the treated fruit of the first pickings ripened in 15 to 18 days, while the untreated lots failed to ripen in 30 days and the condition of the fruit at that time was such as to indicate that ripening would be delayed indefinitely. Later pickings showed decreasingly smaller differences in the ripening time between treated and untreated lots. Post-mature samples, however, picked three weeks after the commercial season still showed seven days' difference between treated and untreated lots.

Effect upon respiration

The increase in the rate of ripening of the treated fruit was accompanied by a marked and rapid increase in respiration (figs. 1, 2, 3). The gases evolved by ripe pears inclosed with the unripened fruit had the same effect in stimulating respiration as did ethylene added to the containers. In the

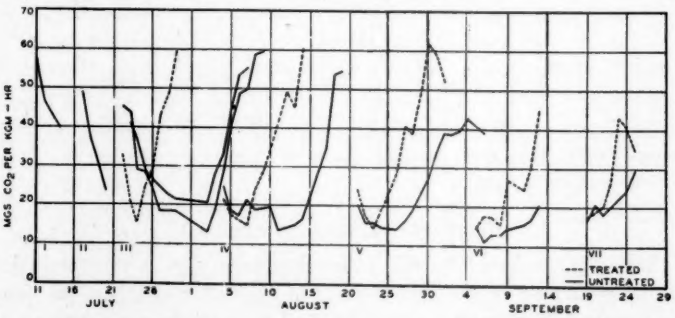

Fig. 1. Effect of gases produced by ripe pears upon the respiration of Bartlett pears picked at different stages of maturity.

untreated lots no similar increase in respiration occurred until after a delayed period of time. The length of this delay, however, was considerably longer with early- than with late-picked fruit, and appeared, therefore, to be associated with the maturity of the fruit at the time of removal from the

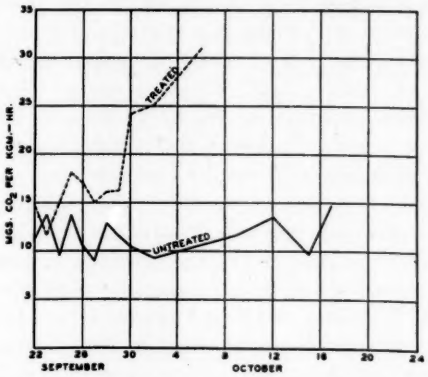

FIG. 2. Effect of gases evolved by ripe pears upon the respiration of Anjou pears treated immediately after picking.

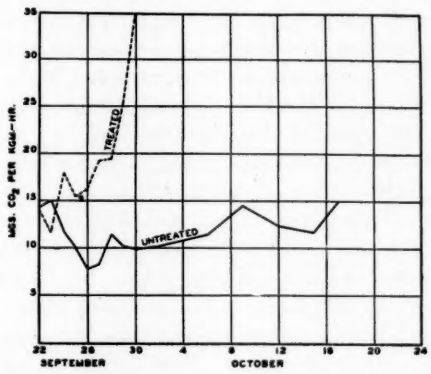

FIG. 3. Effect of gases evolved by ripe pears upon the respiration of Comice pears treated immediately after picking.

tree. Thus in lot III, an early picking of Bartlett, a period of 12 days elapsed in the case of the untreated fruit before an increase in respiration occurred. With fruit gathered at later dates, however, the period between the time of picking and the increase in respiration accompanying ripening became progressively shorter and shorter until with the post-mature lot, the respiratory rate rose rapidly and almost immediately after picking.

It appears, therefore, that fruit in advanced stages of maturity is already at a stage where respiration is tending toward a high rate. Naturally with these pears, little effect from treatments to stimulate respiration can be obtained. Fruit in earlier stages of maturity, however, is either at a stage of low respiratory activity or is tending to drift from a high to a low rate. With these fruits, where the period of low respiratory activity is considerably extended, treatments to stimulate respiration can be expected to be most effective.

As long as the fruit remains attached to the tree, the increase in respiration appears to take place very slowly, and consequently it is considerably beyond the commercial season before the period is reached when pears will no longer be affected by ethylene or naturally occurring gases. Fruit picked during the commercial season was still respiring at a comparatively low rate at the time of picking and showed a marked effect from treatment.

That the effect obtained from ethylene treatment bears a relationship to the maturity of the fruit has also been observed by other workers. WORK (**16**), for instance, obtained the greatest effect from treatment of tomatoes picked 30 to 40 days from blooming. DAVIS and CHURCH (**1**) observed that the stimulative effect of ethylene on the respiration of Japanese persimmons declined as the fruit ripened, especially in the last stages and in the stored fruit. With bananas, HARTSHORN (**8**), using acteylene, found that treatment appeared to be most effective in shortening the initial period of low activity in respiration and consequently the greatest differences were found in those lots in which this period is of greatest duration. None of these workers, however, have associated these differences in responses to ethylene treatment with natural progressive changes in respiration occurring in the fruit during maturation.

KIDD and WEST (**9**), working with apples, first designated the period of rapid increase in respiration as the "climacteric" and have observed (**11**) that while the emanations from ripe apples have the property of hastening the onset of the climacteric, such volatile products were ineffective during the post-climacteric period when softening of the flesh and other outwardly indicated ripening processes occurred. With pawpaws, however, WARDLAW and LEONARD (**15**) have reported that coloring, softening, and final maturation of the fruit are coincident with the climacteric rise. MAGNESS and BALLARD (**13**) have similarly found that the respiration of Bartlett pears increases rapidly as the fruit ripens. The respiration curves of these writers do not show such an extended low level, however, as has been observed in these experiments. This can probably be explained on the basis that in their experiments a period of 3 to 5 days elapsed between the time of picking and the initial run, and during this delay the fruit had probably been subjected to conditions favorable for ripening. It was observed, however, that early-

picked fruit had a lower initial rate of respiration than late-picked samples, and also that the speed with which respiration rate increased was less rapid in the early-picked than in the late-picked fruit.

Comparative evolution of ethylene at different periods

In relation to the differences in results obtained with fruit picked at various stages of maturation, it will be interesting to know at what period the greatest production of volatile substances affecting ripening occurs. Kidd and West (**10**) have stated that the evolution of the effective gas is associated with the climacteric in apples and bananas, but later observations (**2, 6, 12**) have indicated that the pre-climacteric fruit also shows indications of ethylene production. If ethylene is a normal respiratory product in some fruits and plant tissues and shows concomitant increases or decreases with carbon dioxide production, then there are two stages in the life of the pears when evolution of ethylene should be greatest. The first would be in the premature fruit when the rate of respiration is high and the second during ripening when CO_2 production is also maintained at a high level. That there is not a comparable evolution of ethylene at these two periods, however, was indicated by these experiments.

While the presence of ethylene, as indicated by leaf epinasty, could be detected in the premature fruit, the epinastic effect produced by an equal weight of fruit respiring at the same rate, but during the climacteric was strikingly more pronounced. This observation seemed to hold true not only for Bartlett pears, but for the other varieties studied as well. The indications are, therefore, that the evolution of ethylene during the life of the fruit is greatest during the period shortly preceding and including ripening, and is not necessarily concomitant with carbon dioxide production at other periods. Definite proof of this statement, however, must await the development of an accurate method for the determination of small amounts of ethylene.

Since even immature fruit produced a small amount of ethylene as indicated by leaf epinasty, the indications are that no lot of fruit under observation was ever entirely free from the effects of the gas produced within the tissues. In view of this, it appears that the determination of the physiological behavior of fruit entirely isolated from the presence of ethylene will be extremely difficult. The results of these studies indicate, however, that this gas naturally evolved is an important factor to be considered in the respiration and ripening of fruit. The fact alone that there is apparently a very marked increase in the production of ethylene at the beginning of the climacteric would suggest this possibility. Furthermore, the fact that respiration was retarded and ripening was delayed in those lots kept free from an accumulation of any gases by constant aeration, whereas respiration

was increased and ripening hastened in those lots where the gases were allowed to accumulate, strongly indicates that the gas naturally evolved by the fruit, while not necessarily indispensable to ripening processes, does markedly hasten the period when ripening occurs.

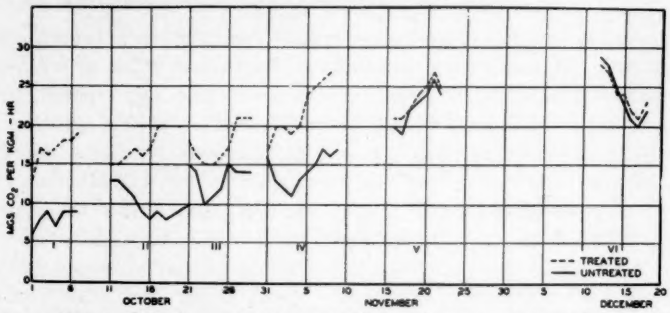

FIG. 4. Effect of ethylene upon the respiration of Anjou pears treated at 65° F. after being held at 37° F. for various periods.

II. How long after picking can an effect from ethylene be obtained?

METHODS.—Since pears are comparatively short lived at high temperatures, it was difficult to determine at just what stage after picking the fruit ceased to respond to ethylene, although the data obtained indicated that treatment was ineffective after the rapid rise in respiration had been nearly completed. In order to follow the changes in response to ethylene treatment after picking more closely, it was decided to use a variety that would show a response over the greatest possible period of time and to further extend this period by the use of cold storage. For this reason, the Anjou pear, a long-lived variety which keeps well under cold storage conditions, was selected. The fruit was picked at commercial maturity from a single tree, and at the time of gathering was in the pre-climacteric stage. Immediately after gathering, two lots were carefully selected for uniformity of size, packed in oil wraps, and stored at 31° and 37° F. respectively. At approximately 12-day intervals, a sample consisting of 30 pears was withdrawn from each lot, taken to the 65° F. ripening room, and treated as indicated in the general methods. As a comparison to the Anjou pear, some studies were also made on Bosc and and Comice, which are shorter lived varieties. The method of handling was the same as outlined with the exception that the 37 degree storage lots were not included.

Effect upon respiration

The period during which the Anjou pears were under observation extended from September 16 to December 12. During this time respiration

data were obtained on six lots removed from storage at consecutive dates. The curves in figure 4, therefore, show, over a fairly long period, the general trends in respiration during ripening of both treated and untreated lots.

That the effect of ethylene upon the rate of respiration steadily decreases after storage is clearly indicated by the results obtained. References to figure 4 show that while there is a very pronounced difference in the rate of

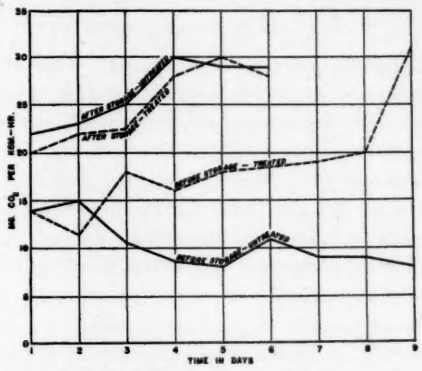

FIG. 5. Effect of ethylene upon the respiration of Comice pears treated before storage and after 6 weeks' storage at 31° F.

respiration between treated and untreated lots after removal from storage early in the season, the magnitude of this difference gradually decreases during a period of two to three months until a point is reached where respiration of the untreated fruit is maintained at as high a level as that of the treated. This period when respiration is no longer affected by ethylene treatment was reached in nine weeks by pears stored at 37° F., while approximately one month longer was required for the pears stored at 31° F. With Comice (fig. 5) no response could be obtained after six weeks, while Bosc were unaffected after only two weeks of cold storage.

Further reference to figure 4 shows that the increase in the rate of respiration, which can be obtained from ethylene over that of untreated lots, is directly correlated with the initial rate or level at which the fruit is respiring at the time of treatment. When this level is low a large increase can be obtained, but when the level is high the difference is correspondingly reduced. Thus, the initial rate of respiration of the first lots removed was at a comparatively low level. The greatest increase was noted in these lots. On subsequent dates, however, there was a gradual increase in the level of respiration of the fruit after removal from storage which was accompanied by a corresponding decrease in effect from ethylene treatment. This trend continued until a point was reached where the fruit, after removal to ripening temperature, was respiring at a high rate, and with these lots no further

increase above the already high rate could be obtained. It appears, therefore, that the rate at which the fruit can carry on respiration is limited to a definite maximum, over and above which no increase can be obtained with ethylene treatment. Furthermore, it appears that there is a gradual drift in respiration toward this high level whether the fruit is held at high temperatures or in cold storage.

Effect upon rate of ripening

Evidence that the effect of ethylene upon the rate of ripening steadily decreases after storage is shown by the differences in ripening rate between treated and untreated lots removed at the different periods. As shown in table II, of the fruit stored at 37° F. for two weeks, the untreated lot required 17 days to ripen, while the corresponding treated lot ripened in 10 days. After a period of 9 weeks, however, the untreated fruit, upon removal from storage, ripened as rapidly as the treated fruit. Similar results were obtained with the 31° F. series, but with these pears, there was still a slight difference in the number of days required for ripening of treated and untreated fruit after 12 weeks. With Comice, after six weeks' storage, the untreated lots ripened as rapidly as the treated, while Bosc showed no response after two weeks of cold storage.

It might be expected that this observed decrease in the time required for ripening could be due to progress in ripening of the fruit while in storage. On the contrary, the indications were that ripening processes, as outwardly manifested at least, had preceded very little during this period. From all appearances, the fruit which showed no response to ethylene was still nearly as much in an unripened condition as those samples which were markedly affected by ethylene treatment earlier in the season. At the time

TABLE II

Effect of ethylene upon respiration and ripening rate of Anjou pears at 65° after removal from storage at 37° F.

No. days of storage	Pressure test at time of removal	Initial rate of respiration Mg. CO_2/Kg./Hr.		Pressure test after 9 days' treatment		Days to ripen	
		Treated	Untreated	Treated	Untreated	Treated	Untreated
12	19.0	13.63	6.74	3.1	13.9	10	17
24	18.7	15.94	13.53	2.5	8.1	9	15
36	17.8	18.74	14.74	2.5	6.1	8	12
48	17.3	17.09	16.71	2.5	3.7	7	10
60	17.0	20.50	20.00	2.0	2.3	6	6
90	8.5	28.00	29.00	2.0	2.0	5	5

of picking the pressure test of the fruit, an indication of firmness or flesh, was 19 pounds. The pressure test of the first lot failing to respond to treatment was still 17 pounds, indicating that very little softening of the fruit had occurred during storage. The change in the fruit, then, that is correlated with the decrease in effect from ethylene, must be accounted for on another basis.

In table II are shown the trends in initial rates of respiration, the number of days required for ripening, and the pressure tests before and after ripening for both treated and untreated lots of the 37-degree series. As previously pointed out, the initial rate of respiration of the untreated lots, upon removal from cold storage, shows a progressive increase. The number of days required for ripening and the pressure test taken nine days after removal from storage show a steady decline in the untreated lots. The pressure test of the unripened fruit before treatment shows very little decrease for nine weeks, but subsequently a very sudden and marked decline occurs. The striking fact brought out is the correlation existing between the initial rate of respiration and the ripening rate of the fruit. Thus, the period when the fruit has nearly attained its maximum initial rate of respiration upon removal from storage, almost exactly corresponds to the period when the fruit ceases to respond to ethylene treatment. This would indicate that, with these storage pears, the effects of ethylene were confined to the period during which the maximum level in respiration was being attained. Furthermore, indications are that the ripening processes including softening of the flesh, occurring after the peak in respiration had been reached, were not affected by the addition of this gas to the ripening chambers.

That the respiration of pears after delayed periods of cold storage starts off at a markedly higher rate than does that of similar fruit before storage, has been previously shown by MAGNESS and BALLARD (**13**). These writers also found that an increase in the respiration of Bartlett pears occurred while the fruit was held at 37° F., but no increase was noted with similar fruit held at 31° F. GERHARDT and EZELL (**4**), however, have found that Comice pears show a consistent increase in respiration during storage at 31° F.

EFFECT OF ETHYLENE UPON PEARS HELD AT COLD STORAGE TEMPERATURES

The results of preliminary investigations, the data of which are not herein included, indicate that ethylene has very little effect upon ripening or respiration of pears held at cold storage temperatures. Bosc, Comice, and Anjou pears treated for periods of from one to three months at 31° and 37° F. showed no apparent increase in respiration or rate of ripening over that of the untreated lots. The indications are, therefore, that the slow progress of the climacteric at low temperatures is not influenced by an increase in the concentration of ethylene over that naturally produced within the fruit tissue.

These results are in agreement with those of KIDD and WEST (**12**) who have observed that the respiration of apples was not increased at cold temperatures upon exposure to the vapors produced by ripe fruit. DUSTMAN (**3**), however, has reported increased rate of ripening of Ben Davis, Rome Beauty, and Stayman apples treated with ethylene for one month at cold storage temperatures.

Discussion

The time of ripening in relation to the occurrence of the climacteric is obviously different with pears held under cold storage conditions than with fruit held at higher temperatures. It will be recalled that with newly-picked pears, ripening symptoms were first apparent with the start of the climacteric; and before the peak of respiration had been reached, the fruit had become fully ripe. With storage fruit, however, while apparently the climacteric occurred at cold temperatures, ripening was delayed until the post-climacteric period or until the fruit was removed to temperatures favorable for ripening. Since the factors associated with the climacteric rise are not clearly understood at the present time, an explanation for the differences observed is not readily apparent. It is clearly indicated, however, that fruit picked in a stage of low respiratory activity tends to drift toward a high maximum rate whether held at high or low temperatures, and while the increase in respiratory activity may occur independently of the ripening of the fruit, activation of the ripening processes appear to be closely associated, at least, with the incidence of this period.

The results obtained with both pre- and post-storage pears indicate that the effects of ethylene are definitely confined to a period preceding the stage in the life of the fruit when the maximum level in respiration has been reached. With fruit treated immediately after picking, the greatest response from treatment was obtained with those fruits having the greatest length of time elapsing before the climacteric, while the least response was obtained in those cases when at the time of picking this rise in respiration had started or was well under way. The results obtained with fruit treated after delayed periods of storage clearly show that ethylene treatment is effective only during the period of ascending respiratory activity, and the magnitude of the response obtained bears a direct relationship to the increase in respiration that has naturally taken place prior to treatment.

The marked differences observed in the periods of time during which long- and short-lived varieties of pears respond to ethylene treatment can be cited as further evidence that ethylene tratment is effective during only a definite period in the life of the fruit. With the Anjou pears, a long-lived variety, over 12 weeks of storage at 31° F. had elapsed before the fruit ceased to respond to treatment. With the Bosc pear, a short-lived variety,

no effect from ethylene could be obtained after two weeks of storage. An intermediate-lived variety, the Comice, showed no response after six weeks of storage. Furthermore, Anjou pears stored at 31° F. responded to ethylene treatment over a period of four weeks longer than did pears of the same variety held at 37° F. It appears, therefore, that with all the varieties of pears studied, there is a gradual drift toward a period of a maximum level in respiration, and when this stage in the life of the fruit has been reached, ethylene treatment is no longer effective. While external factors such as changes in temperature do retard or accelerate the rate at which this increase in respiration takes place, the general trend followed appears to be controlled primarily by internal factors inherent within the fruit.

Since a trend in respiration during maturation similar to that shown by pears has been observed to occur in a number of other fruits (**5, 9, 14, 16**), it may possibly follow that these fruits would similarly respond to ethylene treatment only during a definite life period. This being true, the wide diversity of results that have been obtained from the use of ethylene as a ripening agent can be at least partly explained on this basis.

Summary

1. The emanations from ripe pears have been found to increase the rate of respiration and ripening of newly-picked Bartlett, Comice, and Anjou pears. The greatest effect from treatment was obtained with fruit picked early in the season, and the least effect with fruit picked at post-mature stages.

2. The production of ethylene, as indicated by leaf epinasty, appears to increase during the period of ascending respiratory activity accompanying ripening of the fruit.

3. No increase in respiration or ripening could be obtained from ethylene treatment of fruit which had been held in cold storage for certain periods. Long-lived varieties responded over a greater period than did short-lived varieties. Pears held at 31° F. responded for a greater length of time than did similar fruit held at 37° F.

4. The decrease in effect from ethylene treatment appears to be associated with a natural increase in the respiratory activity of the fruit after picking.

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EXCHANGE OF ELECTROLYTES BETWEEN ROOTS AND ACID SOLUTIONS[1]

RUTH BEALL

(WITH FIVE FIGURES)

Introduction

This paper presents, in abbreviated form, some results of a study of changes in electrical conductance and in hydrogen-ion concentration of dilute acid solutions exposed to the action of seedling roots of *Lupinus albus* L. Effects of these solutions on the plants are also described.

Materials and methods

Three inorganic acids (HCl, H_2SO_4, and HNO_3) and three organic acids (formic, acetic, and propionic) were tested separately, each at three concentrations, 0.00002 N, 0.00008 N, and 0.00016 N. Expressed as molar fractions these concentration values are M/25,000, M/12,500, and M/6250 for HCl, HNO_3, and the organic acids; and they are M/50,000, M/25,000, and M/12,500 for H_2SO_4. The water used was redistilled from Pyrex glass and had a conductance of approximately 2 millionths reciprocal ohm. For control cultures DOAK'S (**11**) modification of HARTWELL and PEMBER'S nutrient solution was used, as well as H_2O.

Seeds of white lupine were germinated in finely chopped sphagnum and the resulting seedlings were introduced into the test solutions when primary roots and hypocotyls were about 6 cm. and 3 cm. long, the cotyledons being still within the seed coats. After rinsing, four seedlings were introduced into each culture jar. The jars used were 500-ml. Pyrex beakers, each containing 500 ml. of liquid. The seedlings were supported by means of cotton in holes in the jar lids, which were perforated Petri-dish covers of Pyrex glass (**2**). The roots were wholly submerged and the hypocotyls were entirely above the liquid surface. Thus nearly the same area of root surface was exposed to the solution in every case.

These experiments were carried out in a dark-room at the Botanical Laboratory of the University of Pennsylvania. For each set of solutions there were two series of experiments, the temperature for one series being about 22° C. for the mineral acids, and about 23° C. for the organic acids; that for the other series being about 28° C. Temperature fluctuated somewhat in each case, within plus or minus 2°.

[1] This experimentation was carried out with the financial aid of a Lydia T. Morris Fellowship of the Morris Arboretum of the University of Pennsylvania.

Changes in ionic concentration of the solutions surrounding the roots were followed by means of daily measurements of electrical resistance, from which were derived the corresponding specific conductance values, computed for a temperature of 18° C. Daily measurements of hydrogen-ion concentration were made by means of the quinhydrone-titration method, with McIlvaine's buffers (**4**). Since the temperature coefficient of hydrogen-ion concentration is so small as to be negligible in such a study as this (**7**), the pH values were not corrected for temperature. Daily observations concerning the appearance of the plants were also made, particularly with regard to development and visible injury.

In the following subsections, changes in the media will be presented first, distilled water and the several acid solutions being considered in order, and then attenion will be turned to records of growth and of injury to the plants.

Experimentation

Changes in the media

Distilled water.—Figure 1 presents graphically average daily con-

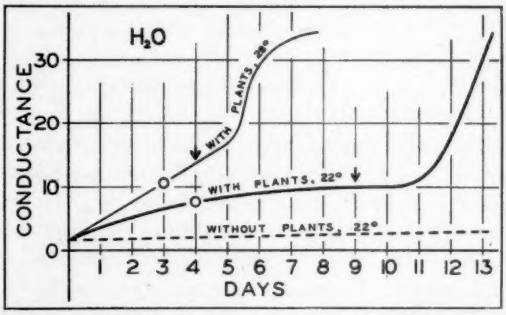

Fig. 1. Conductance (millionths recip. ohm) of H_2O with and without seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

ductance values for H_2O with and without seedlings at 22° C., and for H_2O with seedlings at 28° C. Since no considerable conductance increase occurred without plants, it may be inferred that the increases shown by water with plants were due to outward movement of material from the roots. There was continuous exosmosis of electrolytes, in excess of any absorption that may have occurred. Net exosmosis, which was at first relatively slow, became markedly accelerated about the fifth day at the higher temperature and about the eleventh day at the lower temperature. It was at all times more rapid at the higher temperature than at the lower. At the lower temperature the pH value of H_2O with seedlings increased from 5.4 to 5.7 in

the first day, and then to 6.1 in the following three days, after which it remained practically unchanged. At the higher temperature the changes were very similar, but more rapid, from 5.0 to 5.5 in one day, and to 6.1 in three days. This may indicate either that there was some absorption of hydrogen ion, the effect of which on conductance was overbalanced by relatively greater exosmosis of electrolytes, or that exosmosis acted to reduce the hydrogen-ion concentration of the surrounding liquid.

MINERAL ACID SOLUTIONS.—The manner in which conductance of the mineral acid solutions in contact with seedling roots first decreased and subsequently increased is shown by the graphs of figures 2 to 4. Each graph

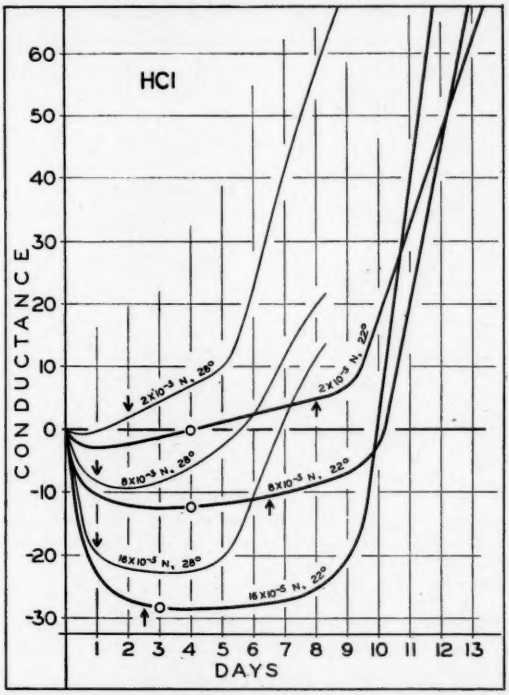

FIG. 2. Conductance change (millionths recip. ohm) in HCl solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

starts at zero on the scale of ordinates (which, in each case, represents the initial conductance of the solution in question) and conductance decreases are plotted as negative while increases are plotted as positive. The three average initial conductances, expressed as reciprocal ohms $\times 10^{-6}$, for each of the three concentrations of each acid were as follows: HCl, 8.9, 26.8, 52.8; H_2SO_4, 8.8, 26.9, 56.2; HNO_3, 8.5, 31.3, 64.5.

Some special measurements made at the end of the first hour showed, in many instances, a slight initial increase in conductance, relatively greater as the original concentration of the solution was higher, which suggests that there may have been an immediate exosmosis of electrolytes from the roots. This detail is not shown on the graphs; it was not seriously studied and warrants no more than mention here.

Each of the graphs shows an initial decrease in conductance, which was very slight and of very short duration for the solutions of lowest concentration, but progressively more pronounced and of longer duration as concentration was higher. Considering the two graphs for each concentration together, it may be said that the period of conductance decrease was shortest

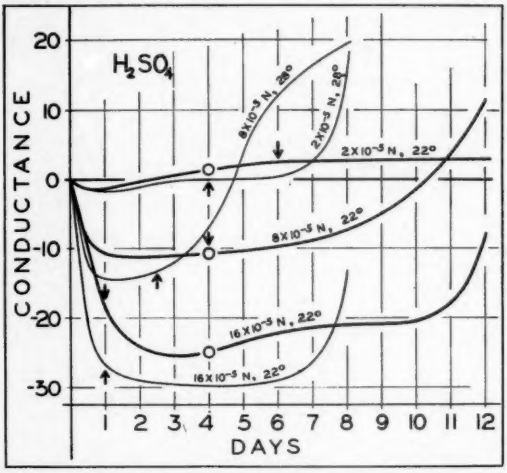

FIG. 3. Conductance change (millionths recip. ohm) in H_2SO_4 solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

(about 1 day or less) for the lowest concentration, longer (about 2 to 3 days) for the intermediate concentration, and longest (about 3 to 4 days) for the highest concentration. Similarly, the average amounts of conductance decrease were approximately as follows, the unit being 1×10^{-6} reciprocal ohm: for the lowest concentration, 2 units (all three acids); for the intermediate concentration, 11 units (HCl), 13 units (H_2SO_4), and 17 units (HNO_3); for the highest concentration, 26 units (HCl), 28 units (H_2SO_4), and 41 units (HNO_3). It is notable that HNO_3 showed greater conductance decrease at the two higher concentrations than was shown by either of the other mineral acids at these concentrations.

As to the temperature relations of the amount of conductance decrease, the available data permit no satisfactory generalization for all three mineral acids. From the graphs of figure 2 it is clear that the amount of conductance decrease for solutions of HCl was regularly somewhat greater at the lower than at the higher temperature. For the lowest and highest concentrations of HNO_3 (fig. 4) the same temperature relation apparently holds, but for the intermediate concentration of HNO_3 and for all tested concen-

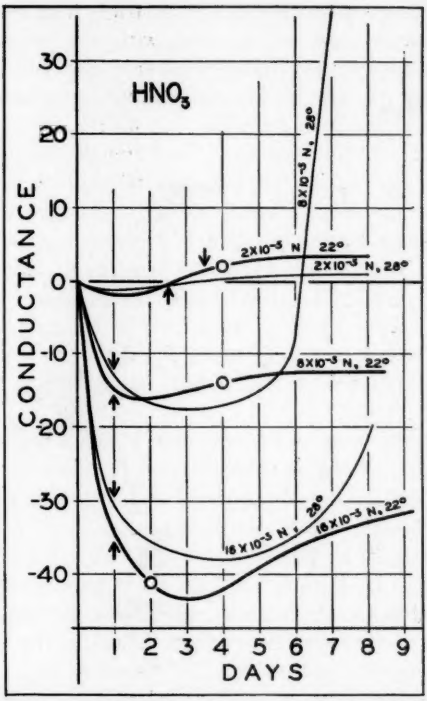

FIG. 4. Conductance change (millionths recip. ohm) in HNO_3 solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

trations of H_2SO_4 (fig. 3) conductance decrease was more or less markedly greater at the higher than at the lower temperature.

When conductance decrease was relatively large in amount its rate is seen to have been rapid at first and then progressively more gradual. For the intermediate and highest concentrations the initial period of decrease was followed by a more or less prolonged period during which conductance was nearly maintained. This period was longer for the lower temperature

than for the higher one. After conductance began to increase, its rate of change sometimes remained low to the end of the experimental period, but in many cases (notably all the graphs of figure 2) its rate was soon rapidly accelerated, so that the final portions of the graphs ascend very steeply.

As to changes in hydrogen-ion concentration, the pH values of all tested solutions—and that of H_2O also—increased from the beginning of the experimental period onward. This increase was most rapid during the first day and became progressively slower till a value of about 6.0 was reached, when hydrogen-ion equilibrium with the roots was apparently established. Eventually the solutions became slightly more basic. The initial pH values of the solutions, corresponding to the initial conductances given above, were as follows: HCl, 4.6, 3.9, 3.6; H_2SO_4, 4.5, 3.9, 3.6; and HNO_3, 4.5, 3.8, 3.5. After one day the following values were reached: HCl, 4.8, 4.6, 5.3; H_2SO_4, 5.1, 4.6, 5.5; and HNO_3, 4.9, 5.1, 5.6. The time required, at the lower temperature, for each medium to attain a pH value of about 6.0 is shown by the position of the circle on the corresponding graph of figures 1 to 4. That time was in general somewhat shorter for the solutions of highest concentration than for the other solutions and for H_2O. The two lower concentrations of the three acids were essentially alike, reaching this value in four days; for the highest concentration the change was somewhat more rapid in HNO_3 (2 days) than in HCl (3 days) or H_2SO_4 (4 days).

Organic acid solutions.—Conductance changes shown by the three organic acids in contact with seedlings are set forth by the graphs of figure 5, which are plotted like those of figures 2 to 4. For each of the three concentrations of each acid, the initial conductance (each plotted as zero on the ordinate scale) was as follows: HCOOH, 6.2, 16.2, 29.3; CH_3COOH, 5.5, 10.6, 16.3; C_2H_5COOH, 4.2, 9.6, 14.1. For each solution the two temperatures tested showed no essential differences in conductance change; the graphs of figure 5 represent the average values for the two temperatures. As in the case of the mineral acids, conductance measurements made within the first day of the experimental period indicated in some instances a very slight immediate increase in conductance, of very short duration, which is not shown on the graphs.

These organic acid solutions showed an initial decrease in conductance, similar to that shown by the mineral acid solutions, but less pronounced. For the lowest concentration of each acid this decrease was of one day's duration or less. It was scarcely noticeable in the case of the most dilute solution of propionic acid, only very slight in the weakest acetic acid solution, and somewhat more pronounced in the lowest concentration of formic acid. In all these cases conductance subsequently increased gradually for about three days, after which it remained approximately unchanged. For

solutions of higher concentration the initial conductance decrease lasted somewhat longer, from one to three days, after which there was, in most cases, some increase, but this was never sufficient to raise the conductance of the solution above its original value. In general, conductance remained unchanged after five days. As was shown for the inorganic acid solutions, initial decrease was greater in amount as solution concentration was greater, the amounts of decrease (in conductance units) being as follows: HCOOH, 2, 11, 20; CH_3COOH, 1, 4, 8; C_2H_5COOH, 0.1, 3, 6.

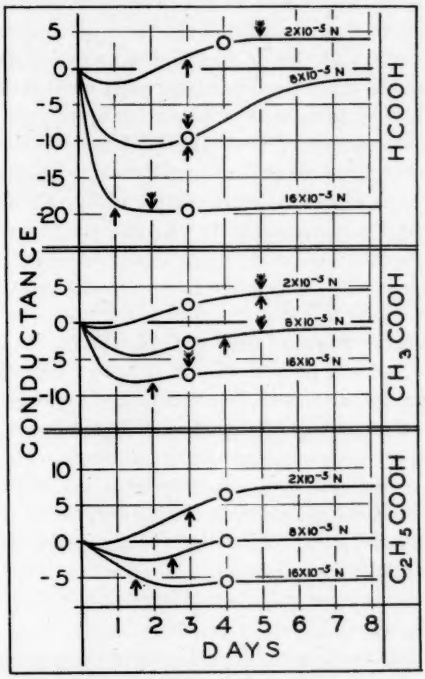

FIG. 5. Conductance change (millionths recip. ohm) in organic acid solutions. Circles mark end of rapid initial increase in pH; winged arrows mark onset of visible root injury at 23° C., naked arrows at 28° C.

Only the graphs for the lowest concentration of acetic acid and of propionic acid and the ones for the lowest and intermediate concentrations of formic acid show any considerable conductance increase within the 8-day period, but of course the conductance of the other organic acid solutions might have increased eventually had the period been longer. To make possible satisfactory comparisons among all six acids, with reference to con-

ductance increase, it would apparently have been necessary to employ experimental periods longer than 8 days.

Turning to the changes in hydrogen-ion concentration of the organic acid solutions, these were very nearly like the corresponding changes that occurred in the mineral acid solutions, except that the original solutions were generally less acid and the amount of change was correspondingly less. The initial pH values of the three concentrations of each of the three organic acids were as follows: HCOOH, 4.5, 3.9, 3.6; CH_3COOH, 4.8, 4.5, 4.1; and C_2H_5COOH, 4.9, 4.5, 4.2. In general there was rapid decrease in acidity during the first day, the following pH values being reached: HCOOH, 5.6, 5.7, 5.8; CH_3COOH, 5.5, 5.3, 5.3; and C_2H_5COOH, 5.3, 4.5, 4.3. Subsequently the decrease in acidity continued, but was more gradual, the solutions attaining a value of about 6.0 by the end of 3 days (intermediate and highest concentration of HCOOH and all three concentrations of CH_3COOH) or at the end of 4 days (lowest concentration of HCOOH and all concentrations of C_2H_5COOH), after which pH was approximately maintained. These times are indicated by the positions of the circles on the graphs of figure 5.

Physiological effects

Controls.—On the roots of the control plants, in nutrient solution, lateral branches always appeared within the first day of the experiment and these subsequently increased in number and enlarged as the primary root continued to elongate. On the second day, when the hypocotyl was well extended, the cotyledons diverged, and later the plumule opened and elongation of the epicotyl began. No chemical injury was apparent and these plants appeared to be quite healthy throughout the entire experimental period of 14 days, excepting that, being in darkness or very weak light, they were markedly etiolated.

Plants with roots in water.—Seedlings with roots in H_2O developed more slowly than those with roots in nutrient solution. Development of lateral roots was similar to that in nutrient solution except that these did not appear until after 2 days and they did not elongate so much. After 2 days also the cotyledons began to diverge and gradually development of the epicotyl proceeded. Injury at the tips of the roots was evident after about 9 days at the lower temperature and after about 4 days at the higher temperature, as is indicated by the arrows of figure 1. Injury to hypocotyls followed in many cases.

Plants with roots in mineral acid solutions.—In solutions of mineral acids development was still more retarded than in water and injury was apparent earlier. Injury was first noticeable as a slight constriction in the primary root, 1 to 2 mm. from the tip. The tip region soon became

flaccid and translucent, then very soft and brown. These changes gradually spread upward until, in the most severe cases, only the upper centimeter or less of the root was apparently free from necrosis. Injury to the hypocotyl usually followed soon after the onset of root injury. It was first evident as a translucent region 1 to 2 cm. long, about the middle of the hypocotyl, and necrosis spread both upward and downward. Subsequently the injured region of the hypocotyl turned brown and collapsed, sometimes with exudation, whereupon the cotyledons and plumules drooped. The plumule developed only slightly or not at all.

Development in the mineral acid solutions was roughly in inverse relation to the original concentration of the solution. Neither primary root nor hypocotyl elongated to any considerable extent in the solutions of highest concentration and both showed less elongation in the solutions of intermediate concentration than in the most dilute solutions. Lateral roots appeared after 2 days in all cases; in solutions of lowest concentration they developed throughout practically the entire length of the primary root, in solutions of intermediate concentration they were confined to the upper two-thirds of the main root, and in solutions of highest concentration they were confined to the upper one-third or less. When the region of their development was restricted they were unusually crowded. At the end of the experimental period they were longer in all mineral acid concentrations than in water, and their length was greater as the region supporting them was shorter. At either temperature, injury to the roots became evident earlier and progressed more rapidly as solution concentration was higher, and for any tested concentration it appeared earlier and progressed more rapidly at the higher than at the lower temperature. At the end of the experimental period injury in the least concentrated solutions was confined to the terminal region of the primary root, in solutions of intermediate concentration it occupied from one-third to one-half of the length of the main root, and in solutions of highest concentration the primary root was visibly injured throughout practically its entire length. Injury to the hypocotyls also appeared earlier and progressed more rapidly as the solution was more concentrated and as the temperature was higher. If the times required for the onset of root injury in these solutions (as shown by the positions of the arrows in figures 2 to 4) are considered roughly as reciprocally proportional to the toxicity of the solutions, then it appears that, for the two lower concentrations at the lower temperature, the decreasing order of toxicity for these three mineral acids was HNO_3, H_2SO_4, HCl. For the highest concentration at this temperature HNO_3 and H_2SO_4 were about alike and produced injury sooner than HCl. For the lowest and for the intermediate concentration at the higher temperature HNO_3 and HCl were about alike and produced injury more promptly than H_2SO_4. Finally, all three acids were approximately alike, on the basis of this time criterion, for the

highest concentration at the higher temperature since all had produced injury within the period before the first examination, but in these cases injury was noticeably more severe in HNO_3 solutions than in the other acids. It thus appears that both temperature and concentration took part —along with the chemical characteristics of the acids—in determining the promptness of the onset of injury.

PLANTS IN ORGANIC ACID SOLUTIONS.—Development was more rapid and more extensive in the solutions of organic acids than in the corresponding solutions of mineral acids. With formic and acetic acids development was somewhat greater in the lowest concentrations than in the others; with propionic, however, development in the solution of intermediate concentration equalled or exceeded that in the most dilute solution, and development in the most concentrated solution was less rapid than in either of the other solutions. By the second day lateral roots had emerged in all cases. They appeared earlier, however (after only 1 day), in the most concentrated solutions of formic acid and acetic acid at the higher temperature, and in all solutions of propionic acid at this temperature. Like the mineral acids tested, the organic acids permitted the development of laterals throughout most of the length of the primary root in solutions of lowest concentration. As the solution was more concentrated laterals were increasingly restricted to the older region of the primary root, but the region bearing laterals was not so limited as under the corresponding influence of the mineral acids. As with the inorganic acid solutions, but to a lesser extent, lateral roots were finally more closely crowded and longer the more restricted was the region from which they arose.

Injury by the organic acids was similar, in inception and nature, to that produced by the mineral acids, but for any tested concentration and temperature it generally appeared somewhat later with the most rapidly injurious organic acid than with the least rapidly injurious mineral acid, as is shown by comparing the positions of the arrows on the graphs of figures 2 to 5. (It should be noted that in figure 5 winged arrows refer to the lower temperature while naked arrows refer to the higher temperature.) Acetic acid generally appears to have been less promptly injurious than either of the other organic acids, which were apparently about alike in this respect. In general, injury by any solution was less prompt at the lower than at the higher temperature and it appeared sooner as the concentration was higher.

THE SIX ACIDS AND H_2O COMPARED AS TO TOXICITY

Although the available data are inadequate for a satisfactory comparative study of the degrees of toxicity of all six acids, it may be of interest to summarize the lengths of time required for the onset of visible root injury, as follows:

1. At 22° or 23° C. the descending order of indicated toxicity for each concentration is:
   a. For lowest concentration: HNO_3 (3.5 days), HCOOH and CH_3COOH (5 days), H_2SO_4 (6 days), HCl (8 days).
   b. For intermediate concentration: HNO_3 (1 day), HCOOH (3 days), H_2SO_4 (4 days), CH_3COOH (5 days), HCl (6.5 days).
   c. For highest concentration: HNO_3 and H_2SO_4 (1 day), HCOOH (2 days), HCl (2.5 days), CH_3COOH (3 days).
2. At 28° C. the descending order of indicated toxicity for each concentration is:
   d. For lowest concentration: HCl (2 days), HNO_3 (2.5 days), HCOOH and C_2H_5COOH (3 days), H_2SO_4 (4 days), CH_3COOH (5 days).
   e. For intermediate concentration: HNO_3 and HCl (1 day), H_2SO_4 and C_2H_5COOH (2.5 days), HCOOH (3 days), CH_3COOH (4 days).
   f. For highest concentration: HNO_3, H_2SO_4, HCl and HCOOH (1 day), C_2H_5COOH (1.5 days), CH_3COOH (2 days).

For each combination of temperature and concentration the reciprocal of the injury-time for each acid may perhaps be taken as an index of its relative toxicity. If for each combination all six reciprocals are expressed in terms of the reciprocal for HNO_3 and if the resulting relative toxicity values for each acid are averaged, the final outcome is a single series of tentative toxicity indices that may represent all the tested combinations of temperature and concentration; that new series is as follows: HNO_3, 1.00; HCl, 0.71; H_2SO_4, 0.66; C_2H_5COOH, 0.63; HCOOH, 0.61; CH_3COOH, 0.41. The corresponding value for H_2O is 0.22. According to these averages, it appears on the whole: (a) that HNO_3 was the most toxic, while (b) CH_3COOH was least toxic of the six acids considered, (c) the former being about 2.5 times as toxic as the latter; (d) that HCl and H_2SO_4 were nearly alike in toxicity (about 69 per cent. as toxic as HNO_3); (e) that C_2H_5COOH and HCOOH were nearly alike in toxicity (about 62 per cent. as toxic as HNO_3), (f) only slightly less toxic than HCl and H_2SO_4, and (g) about 50 per cent. more toxic than CH_3COOH; and, finally, (h) that H_2O appeared to be about half as toxic as CH_3COOH and about one-fifth as toxic as HNO_3. While these statements seem to agree in general with some conclusions that are suggested by superficial scrutiny of the graphs and their appended arrows, it must be remembered that they are intended only to represent a sort of summary of the toxicity phase of the present study, for it is almost certain that the degrees of toxicity of these acids in any test depend on temperature and concentration as well as on chemical nature.

Conclusion

The initial reduction in conductance of these acid solutions was evidently due to absorption of ions by the roots. This absorption was probably accompanied by some exosmosis in all cases, as was shown for H_2O.

While it is conceivable that the conductance of a solution might be diminished by exosmosis alone, if the materials extruded from the roots were of such nature as to remove from solution the ions present, consideration of the nature of the solutions examined in this study and of the kinds of material that might possibly escape from plant roots leads to the conclusion that the observed conductance decrease must have been due to direct removal of ions by the plants. This was no doubt accomplished principally by absorption of material into the cells, but adsorption of ions on to root surfaces may have occurred also. Since solutions of all the acids tested were reduced in conductance, and hence in ion concentration, it seems safe to conclude that ions were absorbed from both inorganic and organic acid solutions.

The amount of net absorption from the several solutions seems to have varied directly with ion supply. For any acid, the higher the ionic concentration of the original solution, the greater and more rapid was the removal of electrolytes. And among the six acids tested, the greater the degree of ionization, in general, the more rapid was net absorption. A general index of absorption from these acid solutions under all the conditions represented by these experiments may be derived by expressing the average amount of net absorption from each concentration of each acid in terms of the average net absorption from the corresponding concentration of HNO_3, and averaging the three values for each acid. This index is as follows: HNO_3, 1.0; H_2SO_4, 0.83; HCl, 0.77; HCOOH, 0.73; CH_3COOH, 0.30; and C_2H_5COOH, 0.12. That is, considering the average of the results of the several experiments, there was greatest absorption from HNO_3 solutions, and least from those of C_2H_5COOH. Net absorption from the organic acid solutions was generally less than from the corresponding concentrations of mineral acids, but HCOOH was only slightly below HCl and H_2SO_4 in this respect. This is in general agreement with the order of dissociation of these acids.

Absorption was, however, apparently influenced by other factors to some extent. Examining the results for the two temperatures separately it may be seen that, for the several mineral acids at the lower temperature, net absorption from HNO_3 solutions was greatest and that from H_2SO_4 solutions was least, net absorption from HCl being intermediate. This order of penetration ($HNO_3 > HCl > H_2SO_4$) is in agreement with DAVIDSON and WHERRY'S (**9**) results with wheat seedlings. On the other hand, in the present study the order of penetration at the higher temperature is: $HNO_3 > H_2SO_4 > HCl$; this is the order reported by HIND (**15**) from her experiments with potato tuber. Net absorption was apparently increased by rise in temperature in the case of all solutions of H_2SO_4 and the intermediate concentration of HNO_3, but in the case of the other solutions (namely, all concentrations of HCl and the lowest and highest con-

centrations of HNO_3) net absorption was apparently decreased. DELF (**10**) and SKEEN (**20**) found the permeability of plant tissues to increase with rise in temperature, and STILES and JØRGENSEN (**24**) report greatly augmented absorption of hydrogen ion under similar conditions. ECKERSON (**13**), on the other hand, reports that the effect of rise in temperature on permeability of root cells varied according to the species considered. Studies on this subject reported in the literature have been carried out with a variety of materials and by means of a variety of experimental procedures. It is safe to presume that rates of penetration of materials into plant roots, and rates of absorption, depend on the kind and condition of the roots as well as on temperature and the nature of the solutions from which the absorbed substances are derived.

The pronounced decrease in hydrogen-ion concentration which accompanied the initial decrease in conductance of these solutions may be taken as evidence that there was rapid absorption of hydrogen ion in the early part of each experimental period. As was the case with absorption in general, this was always greater the higher the concentration of the original solution, and greater the first day than in any succeeding day. Absorption of hydrogen ion by plant cells has been reported by many observers (**5, 14, 18, 19, 23**).

The toxicity of the hydrogen ion has long been recognized. About forty years ago KAHLENBERG and TRUE (**17**) pointed out that the toxic influence exerted upon plants by dilute solutions of strong acids was probably largely due to this ion. Since that time BREAZEALE and LECLERC (**3**), HOAGLAND (**16**), and DUNN (**12**), among others, have reported on acid toxicity for plant tissues. As to the effect of such toxicity within plant cells, ADDOMS (**1**) observed that the protoplasm of root hairs injured by an acid medium appeared to have been coagulated.

It seems probable that the toxic effects produced by the acid solutions of the present study were due chiefly to the hydrogen ion. Similar injuries appeared in all experimental plants and this was the only ion, other than the hydroxyl ion, which was common to all solutions. Also the hydrogen ion is more active, and probably more toxic, than any of the anions present.

In general, degree of toxicity followed hydrogen-ion concentration. For any acid the higher the concentration and the greater the ionic absorption therefrom, the more promptly did injury occur, and in the acids as a group it was generally true that the more completely dissociated the acid the more toxic it was. Propionic acid was, however, more toxic than would be expected from its ionic concentration or from the amount of ionic absorption that occurred from its solutions. Apparently the propionic molecule also was toxic. This is in agreement with TRUE's (**25**) idea that the harmful action of formic and acetic acids is due principally to hydrogen ion, whereas that of propionic acid is due chiefly to the undissociated molecule. Other

workers also have attributed the toxicity of organic acid solutions to undissociated molecules (**6, 8, 12**). Even in the case of the acids whose toxicity seems to have been fundamentally due to excess of hydrogen ion, other ions or molecules may not have been without influence on toxicity.

The toxicity of these acids was generally greater at the higher temperature than at the lower. Injury was apparent earlier and the plants became more severely affected at the higher temperature. SKEEN (**21**) also has found toxicity to be increased by increase in temperature in some cases.

Although, as has been intimated, it appears likely that the initial decrease in conductance of the acid solutions was fundamentally due to hydrogen-ion absorption by the roots, there is indirect evidence that other ions and molecules also were absorbed. Furthermore there seems to be no reason to doubt that there was in all cases some exosmosis of ions or molecules, or both. That the initial lowering of solution conductance ceased—generally after 1 to 3 days for the mineral acids, and after a somewhat shorter time for the organic acids—must have been due to decreased absorption, to increased exosmosis, or to both together. Either of these alterations in the rate of movement of material across the root periphery may well have been occasioned by increasing toxic action. Indeed this increased exosmosis has been used as a criterion of injury by some workers (**8, 22**). With the mineral acids excessive exosmosis from the poisoned plants eventually became greatly pronounced, as was shown by increase in solution conductance, but no rapid outburst of electrolytes had occurred at the time the experiments with the organic acids were discontinued. Nor did rapid exosmosis occur within 8 days in solutions of aromatic acids tested in a similar way by MOTHER CHRYSOSTOM (**8**).

In those cases where conductance increased rapidly toward the end of the experimental period, the sudden increase in exosmosis that is indicated may have been due to a corresponding sudden increase in outward permeability of the root cells to electrolytes, such as might result from some sorts of poisoning, or perhaps poisoning may first have caused general protoplasmic coagulation, followed at length by a sudden disintegration. It is possible that the anions or undissociated molecules of the organic acids acted in some way to retard the poisoning process or hinder protoplasmic disintegration.

Summary

1. Roots of *Lupinus albus* were found to absorb electrolytes from very dilute solutions of certain inorganic acids—HCl, H_2SO_4, HNO_3—and certain organic acids—HCOOH, CH_3COOH, C_2H_5COOH. Absorption was greater in amount and more rapid the higher the original concentration of the solution. For any tested concentration it was greater as dissociation was more nearly complete.

2. There was apparently a rapid absorption of hydrogen ion, probably accompanied by some absorption of other ions, of molecules, or of both.

3. Injury to the plants resulted in all cases. This is probably to be attributed to absorbed hydrogen ion in each instance except that of propionic acid, whose undissociated molecules seem to have been largely responsible for the injury.

4. In many cases rapid exosmosis of materials from the roots eventually occurred, indicating increased permeability of cell membranes or rapid disintegration of cell contents.

5. Influence of temperature, solution concentration, and hydrogen-ion concentration on absorption and exosmosis and on growth and development of the seedlings are among the topics discussed.

This problem was suggested by Professor RODNEY H. TRUE, Director of the Morris Arboretum, who maintained a continuous interest throughout the study. In the analysis of the results and in the preparation of this paper the writer received many helpful suggestions from Dr. BURTON E. LIVINGSTON, of the Johns Hopkins University, and from Dr. CHARLES A. SHULL, of the University of Chicago.

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UREASE DISTRIBUTION IN PLANTS: GENERAL METHODS[1]

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Introduction

Although numerous studies have been made on enzymes with the purpose of elucidating the intermediary metabolism of various substances, such as the fats, carbohydrates, and proteins, the metabolic changes undergone by the enzymes, that is, their synthesis, storage, and decomposition have received scant attention. Of course the metabolism of an enzyme could be discussed only superficially until a few years ago, because its composition was totally unknown. The discovery by SUMNER (**22**) that an enzyme, urease, possessing the properties of a globulin, could be obtained in a crystalline state, marked the beginning of a new era in enzyme chemistry. Likewise, the methods developed by LINDERSTRØM-LANG and HOLTER (**13**) for the application of quantitative histochemistry to enzymes, have led away from a study of gross structures to a more intimate study of tissues and even of cells.

The purpose of the present investigation was to follow the changes in the content and distribution of urease throughout the life cycles of the two leguminous plants which, so far as known, are the richest in their content of this enzyme, namely, the soy bean and the jack bean. The most sensitive tests for most proteins are valueless below a dilution of one part in 10,000. Urease can be detected in much greater dilutions than this. Advantage was taken of the catalytic nature of the enzyme to select certain histological staining reagents which would reveal the presence of the enzyme directly within the cells. By means of these reagents it is possible to study the changes of urease activity taking place during the histological development of the plant. Since the number of cells per organ (for example, the leaf) does not change greatly after an early stage in the development of that organ, one can study, by analyzing organs of different ages, the progressive changes in urease content of cells of increasing age.

It was hoped that the data would not only reveal the course of urease metabolism but would also clarify a number of other problems as well. Considering urease as a protein, one can follow the metabolism of a protein of specific constitution throughout the life history of a plant. If the assumption be made that the main portion of the protoplasmic framework of a cell

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This is the first of a series of three papers on urease distribution in *Canavalia ensiformis* and *Soja max*. More extensive data and discussion of this subject may be found in the writer's thesis in the University of Michigan library.

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consists of proteins, it might be possible, by following the changes in urease activity of the cells under various environmental conditions, to get some information about the changes occurring in the protoplasmic framework. Also, since urease has been found in plants of every division of the plant kingdom (**4, 27**), it becomes interesting to determine what fundamental relation the enzyme has to the nitrogenous metabolism of the plant.

The reaction catalyzed by urease, as shown by MACK and VILLARS (**15**) and by later investigators, is the hydrolysis of urea to ammonium carbamate. Urea itself is very slightly ionized. Ammonium carbamate is alkaline; it is unstable, especially in acid solutions, and undergoes spontaneous hydrolysis to ammonium carbonate. Two general methods have been used in this investigation: a histological method which depends on the increase in alkalinity as urea is hydrolyzed by urease; and a quantitative method based on the determination of the ammonia produced.

Histological urease determinations

An attempt was made to find a method which would give direct visual evidence of the exact location of urease in the tissues. WAGENAAR (**25**), SEN (**20**), and WASICKY and KRACH (**27**) used a number of reagents. The last-named investigators found that reagents for the determination of NH_4^+ such as Nessler's reagent, chloroplatinic acid, etc., were much less sensitive than pH indicators, and suggested the use of an alcoholic haematoxylin solution as a suitable indicator. After testing a number of methods, the writer came to the same conclusion as WASICKY and KRACH, namely, that the change of hydrogen-ion concentration was the most sensitive method upon which to base the detection of urease.

Two histological methods were used, the indicator and the lake methods, both of which depend upon the detection of urease by an increase in alkalinity.

INDICATOR METHOD

A number of factors which would influence the sensitivity and accuracy of the indicator method had to be considered. These may best be appreciated by imagining an enzyme point in a cell at which urea molecules are undergoing decomposition. The first molecules of ammonium hydroxide released at this point will react with various buffers in the immediate vicinity of the enzyme point. This ammonium hydroxide will therefore not show up as a change in hydrogen-ion concentration and the indicator will not be affected. After sufficient ammonia has been produced the pH of that region will shift toward the alkaline side. The rapidity of the pH change will then partially depend on buffer capacity. It is readily seen that the most sensitive indicator to choose will be one whose midpoint (pK) will be that of the

hydrogen-ion concentration of the cells. It is therefore necessary to know the hydrogen-ion concentration of the tissues examined. The series of indicators recommended by SMALL (**21**) was found suitable for an approximate determination of the pH of the tissues.

INDICATORS USED.—Because of their visually favorable change in color from yellow at the acid end to dark blue at the alkaline end, the indicators brom-cresol purple, brom-thymol blue, brom-cresol green, etc., were used in a 0.2 per cent. aqueous solution to detect enzymic action. They were adjusted to their midpoint with 0.05N acid or alkali. Although these indicators showed the presence of the slightest traces of enzyme their colors were not intense, but quite diffuse, so that only a vague notion could be obtained as to the enzyme location. Haematoxylin, on the other hand, was found to be excellent for the purpose. Saturated aqueous solutions of haematoxylin were prepared fresh every three or four days since changes take place in this indicator on standing. It is yellow at the acid end and intensely red at the alkaline end of its range, with a midpoint around pH 6.5. Besides showing a marked color change with change in pH, the dye in its alkaline form appears to be quite localized in a tissue. It was found upon further study that the permeability of the cells is much less to alkaline haematoxylin than to the neutral or acid haematoxylin. The yellow-colored (acid) haematoxylin could be washed out readily with water, without as readily removing the red-colored haematoxylin.

PERMEABILITY OF CELLS TO INDICATORS AND UREA.—If one is to judge the location of the enzyme by a change in color of an indicator within the cells, one must know that the indicator and urea solutions have penetrated all the different cells of a tissue section. The penetration of the indicator is quite readily determined by noting its distribution. Generally, the dye colors all of the cells of a tissue section. However, in certain instances, as, for example, in sections of the collet region of jack bean seedlings, some pith cells appear to be less readily permeable to the dye than their neighbors. In such instances, the dye solution is allowed to remain on the section for a few minutes longer until these cells also are colored.

It has generally been considered that cells are readily permeable to urea. HÖFLER and STIEGLER (**8**), HÖFLER and WEBER (**9**), and WEBER (**28**) have shown, however, that the penetration of urea into the cells of various tissues of the same plant and even of the same tissue was different; permeability toward urea was also found to differ with the age and the activity of the cells. By treating cells of various tissues with 20 per cent. ethyl alcohol in order to destroy the plasma membranes and permit unhindered diffusion of urea into all of the cells, it was found that the controls and alcohol-treated sections showed no marked differences in permeability toward urea. In a few instances the alcohol-treated sections appeared to contain slightly less

enzyme, but the distribution even in these sections was the same as in the controls. Perhaps even the least permeable cells allowed sufficient urea to enter from the aqueous urea solution. SMALL (**21**) reports an increased permeability and outward diffusion of salts from cells treated with 20 per cent. alcohol. The enzyme apparently is not rapidly destroyed in 20 per cent. alcohol. Indeed, sections of jack bean cotyledons immersed for a few minutes in a 95 per cent. alcoholic solution still possessed active urease.

BUFFER CAPACITY OF CELLS AND REAGENTS.—As already indicated, it is necessary to take into account the buffer capacity of the cells studied because, if ammonia is released at the enzyme points, the buffers of the cell will neutralize the ammonia; the rate of indicator change toward the alkaline side will therefore depend upon the buffer capacity of the cell. Although one cell may have a greater enzyme content than another, yet the amount of enzyme in the first cell may be masked by its greater buffer capacity. Likewise, it is possible that a cell may possess both the enzyme and a high buffer capacity, whereas a neighboring cell may contain no enzyme but have a low buffer capacity; in this instance ammonia would diffuse to the neighboring cell containing no enzyme and change the indicator to the alkaline side and apparently indicate the presence of the enzyme. It was found that the meristematic tissues, including the cambium, are most highly buffered, next come the smaller parenchyma cells, then the larger parenchyma cells of the cortex and pith, and finally the xylem cells.

At first glance it would seem that, because of the impossibility of controlling the tissue buffers, the indicator method would be useless for the exact localization of the enzyme in specific cells and tissues. Fortunately, this is not generally so. In sections of the apical bud, where buffer capacity must be considered, it is found that the apical primordium and the axillary primordia, which are the most highly buffered tissues, are also the tissues containing the most enzyme. On the other hand, the buffer capacity in the cambium does not permit a comparison of the enzyme content of that tissue with the content of neighboring parenchyma cells. This is true in the stem below the third node (first node above the cotyledonary node) of the seedlings. In these parts, the enzyme content of the pith and cortex is relatively high, but none can be detected with certainty in the cambium or its derivatives. Above the third node, however, the pith and cortical parenchyma contain no enzyme detectable by the indicator method, and the cambium also gives a negative reaction. That the cambium and its derivatives contain no urease is a generalization that may be made, while keeping the limitations of the indicator method strictly in mind.

Since the enzyme is detected by a change in the hydrogen-ion concentration, it is essential that the reagents used should also have little or no buffering power. Determinations of the buffer capacity of the 0.2 per cent.

dye solutions and the 1 per cent. urea solution showed it to be very slight in these solutions. As contrasted with the buffer capacity of any of the parenchyma cells examined, that of the indicators and of the urea solutions is negligible.

TIME FACTOR.—It is evident that if urease is permitted to act for a longer time more ammonia will be formed and the tissue will become more alkaline. The maximum time for observation is limited by the following considerations: In the first place, carbonic acid is constantly being produced, and neutralizing a portion of the ammonia released. In certain tissues, the enzyme content is so slight that more CO_2 than ammonia is produced and the acidity of the tissue increases, as has been determined in a few experiments with a sensitive glass electrode apparatus. In the second place, the enzyme appears to be slowly inactivated. Thirty minutes was found to be the maximum time that could be used for these histological determinations.

TISSUE SECTIONS.—For approximate estimates of the enzyme, sections were made with a hand razor. If delicate structures were to be examined or accurate comparisons of sections were to be made, a freezing microtome was used at first. It was soon found, however, that small pieces could be imbedded in paraffin of low melting-point and rapidly cooled, without appreciable destruction of the enzyme. The time, from removing the material from the plant until the section was ready for study under the microscope, varied between 15 and 20 minutes.

LAKE METHOD

Although a particular indicator will permit the detection of urease by the first appearance of color change, the ammonia generated at the enzyme points diffuses so rapidly into the surrounding cells that the color of the alkaline indicator becomes quite diffuse. The ammonia may also invade tissues containing no enzyme and the indicator test may then give spurious results. It is therefore necessary, for sharp localization of the enzyme, to secure a reagent which will form a non-diffusible precipitate at the enzyme points. At the same time it is desirable to poison the enzyme points in order to prevent ammonia from being continuously liberated and invading the neighboring tissues. It is necessary, moreover, that the precipitate be readily visible within the cells. Of the large number of reactions tested, the formation of a lake (an adsorption complex of a dye with the hydroxide of a heavy metal) satisfies these three requirements.

The inactivation of a urea solution with various metals has been studied by SCHMIDT (**19**), and more recently by JACOBY (**11**). The following was found to be the order of poisoning effectiveness: $Cu^{++} > Hg^{++} > Ag^{+} > Ni^{++} > Fe^{+++} > Zn^{++} > Pb^{++}$. The last elements had only comparatively slight effect on urease activity.

The formation of metallic hydroxides at various hydrogen-ion concentrations has been studied by BRITON (3) and others. They find that the hydrogen-ion concentrations at which the hydroxide will form is a characteristic of that metal. For example, Ni^{++} will form its hydroxide when added to a solution whose hydrogen-ion concentration is pH 6.7 or above. The hydroxides of the heavy metals are generally very insoluble gelatinous precipitates which would be difficult to discern when formed within the cells. With the proper dye adsorbed, it is possible to detect traces of metallic hydroxides.

The application of the principle outlined is best illustrated by a specific example. Microtome sections of an apical meristem are treated with aqueous haematoxylin. This is allowed to remain on the sections for a few minutes until it has penetrated all of the cells. Excess fluid is drained off and a drop of a 1 per cent. urea solution and a drop of 0.05 M $NiCl_2$ are now added. A cover slip is placed over the sections, excess fluid is drained off, and the slide is examined immediately with a microscope to note the distribution of the deep blue color of the nickel hydroxide-haematoxylin lake.

The order of adding the reagents depends on the amount of enzyme present. If the enzyme is highly concentrated, as it is in the mature cotyledons, it is advisable to use a solution of nickel chloride and in addition a trace of cupric or mercuric chloride to poison the enzyme more rapidly. This solution is added immediately after the urea. If, on the other hand, the enzyme content is very low, it may be desirable to add a very dilute nickel chloride solution some time after the urea solution is added. The enzyme action will convert the yellow haematoxylin to a red-purple color. On addition of the dilute nickel chloride solution, this color will become deep blue and quite sharply delimited from the yellow of the surrounding tissues.

Quantitative urease determinations

An excellent discussion of various quantitative methods for the determination of urease is given by EULER (4). After several methods had been tested, a colorimetric method was chosen. The ammonia produced by a piece of tissue containing urease, after the latter has acted on a urea solution for a certain time, is driven over with a rapid current of air into an acid solution in which the ammonia is determined. With some modifications the method is essentially that described by VAN SLYKE and CULLEN (29).

A weighed portion of fresh tissue is finely ground in a porcelain mortar with 1 to 2 cc. of M phosphate buffer (pH 7.2), with quartz sand when necessary. The mixture is transferred to a volumetric flask of appropriate capacity, made up to volume with distilled water at 30° C., and the flask placed in the water bath (30° C.). Into each of the several reaction tubes

are pipetted 1 cc. of phosphate buffer, 1 cc. of freshly prepared 2 M urea solution, and the requisite amount of distilled water. The amount of distilled water is so chosen that the final volume of solution in the reaction tube is exactly 10 cc. The tubes are also placed in the water bath. After they have come to the temperature of the bath, an aliquot of tissue solution is pipetted into each tube. The time from the beginning of grinding until the tissue is added to the reaction tube varies between 10 and 15 minutes. Exactly 30 minutes (stop watch) after the tissue is placed in the tube the urease is inactivated by the addition of 0.5 cc. of 2.5 N H_2SO_4. The acid solution serves a twofold purpose. It not only inactivates the enzyme solution completely, but also decomposes the ammonium carbamate which was produced by the hydrolysis of urea. (Alkali does not inactivate urease completely.) After 5 minutes, the reaction tube is placed in the aeration apparatus. A few drops of caprylic alcohol and 5 gm. of anhydrous K_2CO_3 are rapidly added, the tubes stoppered, and suction from the water pump is applied. Control tubes containing tissue but no urea are treated in all respects like those described above. After all the ammonia has been driven over into the tube containing acid, it is determined colorimetrically with a Duboscq colorimeter. If the ammonia content is above 0.05 mg., the customary Nessler's reagent is used. For determinations of less than 0.05 mg. NH_3, the sodium phenate reagent as modified by VAN SLYKE and HILLER (**30**) was used.

The sum of the ammonia present in the tissues and in the reagents, subtracted from the total ammonia values obtained, gives the ammonia produced through the decomposition of urea by the urease of the tissues.

Using a maximum of 0.5 gm. of tissue and a time-period of 30 minutes for reaction the method permitted the determination of a wide range of enzyme activity. In actual practice, the enzyme values ranged from 30 U.U. down to 0.0001 U.U. where ***U.U., the urease unit, is a quantity of urease which will produce one milligram of NH_3 per minute under the experimental conditions employed.***

FACTORS CONCERNED IN QUANTITATIVE UREASE DETERMINATIONS

The quantity of urease is determined indirectly by measuring the rate of formation of ammonia from urea under certain standardized conditions. These conditions were chosen and various other factors examined in relation to their influence on urease activity. Although numerous publications have dealt with the quantitative measurements of the activity of a particular tissue containing an enzyme few bear upon the difficulties that arise in an attempt to make comparisons of the quantity of enzyme in the same tissue at different stages of development, or in different tissues. A number of factors cannot be adequately controlled. However, an approximation to the true values of enzyme activity can be obtained.

A number of methods have been used by investigators to prepare the plant material for enzyme analysis. For determinations of the total enzyme content it is obviously useless to extract the enzyme, since extraction is never complete; it is different for different materials; and during extraction inactivation of the enzyme occurs.

EFFECT OF DRYING.—It is important to learn whether tissues may be dried without causing changes in enzyme activity. PETT (17) has studied the effect of drying germinated wheat seeds and concludes that the dipeptidase activity increases on drying. Since he used a glycerol extract of the material for his enzyme determinations, it may be that drying merely increased the amount of enzyme extracted by the glycerol, and not the dipeptidase activity.

In the following experiments the material was spread out on filter paper and allowed to dry at room temperature (approximately 22° C.). The urease content was determined immediately and after several days of drying.

TABLE I

EFFECT OF DRYING ON UREASE ACTIVITY

PLANT MATERIAL	TIME AFTER COLLECTING	MOISTURE	U.U. PER BEAN
		%	
Soy beans, nearly mature (beans removed from pods; 10 used for each determination)	Immediate	61.0	1.01
	1 day	35.2	0.988
	7 days	10.1	0.976

TABLE II

EFFECT OF DRYING ON UREASE ACTIVITY

PLANT MATERIAL	TIME AFTER COLLECTING	U.U. PER GM. OF ORIGINAL FRESH WEIGHT
Jack bean leaflets (young; 5–8 cm. long; 15 used for each determination)	Immediate	0.0542
	6 days	0.0344

The data in table I show only a slight decrease in urease content during slow drying of nearly mature soy beans. In the young leaflets of jack bean (table II), however, air-drying decreased the urease activity 37 per cent. Because of these results it was decided that all analyses should be made on fresh tissues.

EFFECT OF MACERATING.—To determine what effect crushing and macerating the tissues would have on urease activity, opposite leaflets (with petiolules removed) of jack bean plants were used. These leaflets possess numerous small stomata on the under surface but none on their upper surface. Whole leaflets, leaflets cut into 5-mm. squares, and macerated leaflets were compared

as to their urease activity. It was found that with a 0.2 M urea solution the whole leaf showed an activity one-thirtieth that of the macerated tissue. The 5-mm. squares of leaflets had an intermediate value. Infiltration of whole leaflets with urea by means of suction, and increasing the length of time for reaction on the urea solution to 60 minutes, increased the indicated urease values of whole leaflets to one-fourth that of macerated leaflets. It appeared probable that the lower value of urease activity, if whole leaflets were used, was because of the inability of the urea solution to come into contact with all of the available enzyme points in the cells. It was thought possible that the permeability of the plasma membranes might be increased by the addition of 1 and 2 per cent. ethyl ether or ethyl chlorhydrin to the urea solution. Determinations on macerated, as well as on whole tissue, showed, however, that these chemicals, at the concentrations used, inhibited urease activity from 15 to 25 per cent. No increase in urease activity was observed with leaf-tissue mash suspended in 5 per cent. sodium chloride. Another series of experiments was performed to determine whether very high urea concentrations would not give values for urease activity of the whole leaf approximating those of macerated leaves. Using 1 to 4 M urea solutions, it was found that the activity of urease in whole leaves, especially at the highest urea concentrations, approximated that of macerated leaves. It may be concluded that maceration of the jack bean leaves causes no detectable inactivation of urease. In this connection it is interesting to note that LINDERSTRØM-LANG and HOLTER (**13**) report no difference in the activity of peptidase present in whole sections of barley roots as compared with crushed sections.

UREA CONCENTRATION.—An attempt was made to choose a sufficiently high urea concentration so that the amount of decomposition produced in a given time would be independent of the concentration. The data of VAN SLYKE and CULLEN (**29**) and of LÖVGREN (**14**) on soy bean extracts indicate that the rate of decomposition does not increase above a concentration of 2 M urea, although the increase above 0.2 M is slight. Results were obtained on soy bean cotyledons that were in accord with the findings of these investigators; that is, there was no appreciable increase in the decomposition rate above 0.2 M concentration of urea. A concentration of 0.2 M was then chosen for the quantitative investigations. It is a convenient concentration to handle, because a 2 M urea solution can be made up, and 1 cc. of this pipetted into a reaction tube, tissue mash and buffer added, and the whole finally diluted to 10 cc.

It was later found that the conclusion arrived at for the urea concentration of soy bean cotyledons does not hold for jack bean leaf tissue. Even above a 2.1 M substrate concentration there still is an appreciable increase in the rate of urea decomposition. With 0.1 M urea solution, 0.61 mg. NH_3 was produced; with 0.7 M urea solution, 0.68 mg. NH_3; with 2.1 M urea

solution, 0.72 mg. NH_3; and with 3.5 M urea solution, 0.76 mg. NH_3. Obviously, one cannot speak of a maximum concentration above which all the enzyme points are being readily supplied with urea without specifying the tissue-preparation used. One may conclude that, if different tissues or plant parts, such as roots and leaves, are to be compared as to total urease content, an error of 25 per cent. may arise merely because of insufficient urea concentration; that when the same tissues or plant parts are being compared this error is eliminated.

The *temperature* chosen was 30° C. This is sufficiently high temperature to effect rapid enzymic hydrolysis, but not high enough for inactivation of the enzyme to become an important factor.

The *reaction time* was chosen as 30 minutes. This was selected after a number of preliminary trials had been made. Because the rate of inactivation of different enzyme preparations could not be controlled, the shortest time was chosen in which determinations of the lowest enzyme concentrations could be determined accurately. This of course depended on the sensitivity of the method selected for the ammonia determinations.

Some idea of the rate of urease inactivation was obtained from the following experiments. The moment that the maceration of the tissues was begun was taken as zero time. After a thorough grinding and proper dilution, the mash was placed at 30° C. and overlaid with toluene. At various intervals, an aliquot of the mash was taken for analysis, and allowed to react on the urea for 30 minutes at 30° C.

TABLE III

RATE OF INACTIVATION OF MASH CONTAINING UREASE AT 30° C.

MATERIAL USED	TIME AFTER BEGINNING OF MACERATION		U.U. PER GM. FRESH WT.
	min.	*hr.*	
Soy bean cotyledons (germinated 36 hours)	55		3.92
	115		3.86
	295		3.54
Jack bean leaflets (5–8 cm. long)	49		0.0547
	94		0.0550
	194		0.0603
	361		0.0605
		21	0.0574
		140	0.0069
Jack bean leaflets (5–8 cm. long; dried at room temperature 6 days)	66		0.0344
	396		0.0041

From table III it is seen that the decrease of urease activity in the soy bean cotyledons is negligible during the first few hours. It is interesting to note that the mash, made from fresh leaflets of jack bean, actually increases in urease activity during the first three hours. Here again the change of activity, during the first hour after addition of urea, is negligible. With leaflets of jack bean, dried at room temperature for six days, the rate of inactivation appears to be especially marked; no change in urease activity was observed on the addition of 0.5 cc. of saturated aqueous H_2S to the reaction tubes containing an aliquot of mash. These experiments indicate that two simultaneous processes are going on: an inactivation of the enzyme by denaturation, coagulation, or poisoning; and a dispersion, peptization, or activation of the enzyme.

The *hydrogen-ion concentration* of pH 7.2, which was produced by a phosphate buffer, was used for the urease determinations. LÖVGREN (**14**), using phosphate buffers, reported an optimum pH between 7.2 to 7.8 for soy bean extracts. HOWELL and SUMNER (**10**) found that the urease activity depended on the type of buffer and on the concentration of urea, the pH optimum increasing with decreasing urea. BACH (**1**) found an optimum hydrogen-ion concentration of pH 8.6 for a urease preparation extracted from an *Aspergillus*. It is quite possible that the optimum hydrogen-ion concentration for a urease preparation may depend to some extent on its concomitant impurities, as has already been found for certain enzymes. No experiments were conducted to determine whether the hydrogen-ion concentration optimum is different for different structures of the same plant. With the dipeptidases of the wheat grain PETT (**17**) found about the same pH-activity curve for all portions of the wheat grain that were studied.

Since ammonia is produced during urea hydrolysis and the solution becomes alkaline, it is necessary to have a sufficient amount of buffer present to prevent any marked change in the hydrogen-ion concentration. In this investigation, a quantity of enzyme material has been taken which will generally produce less than 1 mg. of NH_3 in 30 minutes. A concentration of 0.1 M phosphate buffer (prepared from Na_2HPO_4 and KH_2PO_4) was used, which when present in the enzyme substrate mixture was found to keep the hydrogen-ion concentration from increasing more than three-tenths of a pH unit.

The *recovery of ammonia with the aeration apparatus* was determined, using standard solutions of ammonium sulphate, and also using mash plus standard ammonium sulphate solutions. Although 90 per cent. of the ammonia was recovered after 30 minutes of aeration at room temperature, recovery was complete only after two hours of aeration. The presence of mashed tissue did not interfere with recovery.

SAMPLING OF PLANT MATERIAL.—Analyses were made on the basis of the fresh weight of plant organs or parts of organs. The organs were removed

from the plants in the morning, then weighed and analyzed immediately. For each analysis generally ten organs were selected from ten different plants. The tissues were ground and duplicate determinations were run on aliquots of the mash, including also a control for the free ammonia present in the mash. In selecting samples for analysis, an attempt was made to choose plants that were similar in number and length of leaves and internodes. Plants either more or less vigorous than the average were discarded.

Discussion and conclusions

It is necessary to clarify the term "quantitative" in reference to enzyme determinations. An enzyme is detected only through the catalysis of a more or less specific reaction. This criterion is, however, not sufficient to establish the absence or the total quantity of enzyme, but merely the amount of "active" enzyme present.

An extensive review by LÖVGREN (**14**) of the inactivation of urease by various substances indicates that inactivation, in most instances, can readily be accounted for by a denaturization or coagulation of the protein. As for activation by specific substances, WALDSCHMIDT-LEITZ and STEIGERWALD (**26**) report that urease activation with glycine or hydrocyanic acid is observed only with crude enzyme preparations, and not with purified or crystalline enzymes. There is some evidence (**2, 5, 7**) that oxidation inactivates and reduction reactivates urease but the meaning of this is not clear.

The data at present available on crystalline hydrolytic enzymes, including urease, favor preponderantly the contention (**24**) that these enzymes are proteins containing in their make-up, and holding by chemical linkages, peculiar arrangements of atoms which constitute the active groups. Since urease can be detected only in its active state, that is, by the number of active groups with which the urea molecules can come in contact, it becomes important to examine under what conditions and to what extent these groups are available for action.

In the cells, proteins are present in various states of aggregation, some protein molecules being monomolecularly dispersed, others clustered into larger groups, and still others aggregated into ergastic protein materials such as the globoids, aleurone grains, and crystals. The protein urease likewise can be considered to be present in the cells in these various states of aggregation. GRABAR and REIGERT (**6**) have recently investigated the activity of urease as related to particle size. Using four different urease preparations they found, by means of membranes of graded porosity, that the particles of urease were in different states of aggregation. Crystalline urease in aqueous solution was the most homogeneous preparation examined, and had dimensions near those of serum globulin. On digestion of the urease preparations with trypsin it was found that those particles small enough to pass through pores 15 mμ in diameter possessed no urease activity. Under the same

conditions serum albumin passed through pores 30 mμ in diameter but not through 9 mμ pores. Crystalline urease was the most active preparation. From these data, it appears that the activity of a urease preparation is at its maximum when the urease particles are of the dimensions of serum globulin particles (mol. wt. 150,000); presumably the urea molecules are now able to come into contact with all the available active groups of the urease particles. The work of KIRK and SUMNER (**12**) may be cited as evidence that the decrease of urease activity occurs with agglomeration of urease particles. These workers noted that the inhibiting effect of anti-urease (antibody) on urease consists, largely but not entirely, in decreasing the dispersion of urease.

From the above, it may be seen that when larger groups of urease molecules, as in the form of ergastic materials in the cell, are dispersed, an apparent synthesis may occur. If urease is decomposed proteolytically below a certain molecular weight there will be a loss of urease activity; but there may also be a decrease in urease activity or apparent decomposition when the enzyme becomes denatured, agglomerated, or stored in some form in which it is not readily dispersed. The urease that is detected is, in the main, in a highly dispersed condition. The data on the rate of inactivation of mash of jack bean leaf show an increase in urease activity during the first three hours after maceration, indicating that more of the active groups have become available than are being removed by autolysis or denaturization.

There is no way at present of determining the total quantity of enzyme present (**16, 18**). The methods used in this investigation have been chosen to determine as nearly as possible the relative amounts of available active groups present in the cells at the time of examination. In all instances, where comparisons could be made of the histological and the quantitative methods with the same tissue, it was found that the results obtained with both methods checked each other nicely. One may infer, therefore, that in the macerated tissue, the enzyme does not differ in activity to any extent from the enzyme in the cells of tissue sections. The experiments in which "whole" and "macerated" leaf tissues were compared showed that macerating caused no detectable inactivation of the enzyme. Further experiments (table III) indicate that urease inactivation within the first 30 minutes after crushing the tissue is slight or negligible; and this has been the length of time chosen for the enzyme to act on urea.

Although, with the cotyledonary tissue of soy bean, there is no increase in the rate of urea decomposition above 0.2 M urea concentration, this is not true for the tissue of the jack bean leaf. In this leaf tissue, there still appears to be an appreciable increase in the decomposition rate of urea even above a 2.1 M concentration. This may be another instance of making the active groups of urease available, since it is known that urea in high aqueous concentrations has a powerful solvent action on many proteins, and appears to split certain

protein molecules. Edestin, for example, is split into particles whose molecular weight is about one-fourth of that determined by SVEDBERG for edestin in neutral salt solutions. For convenience, a concentration of 0.2 M urea was used in these determinations. When different plant structures are to be compared as to total urease content, an error of 25 per cent. may arise because of an insufficient urea concentration; when the same tissues or plant structures are being compared, however, this error is eliminated. Fortunately, the differences of urease content of different structures are much greater than 25 per cent. so that this factor does not influence any of the conclusions that have been drawn from the data.

Although the limit of sensitivity of the quantitative method is 0.0001 U.U. per gram of fresh weight of tissue (where a urease unit, U.U., is that quantity of urease which will produce 1 mg. of ammonia per minute), the histological method is not as sensitive. From comparative determinations it has been found that the indicator method cannot detect urease in tissues having a lower activity than 0.02 U.U. per gram fresh weight.

By making certain assumptions,[3] it may be calculated that there are about 25 urease molecules contained in a cell, at the limits of sensitivity of the quantitative method. Since the indicator method is only one-two hundredth as sensitive as the quantitative method, at least 5000 urease molecules must be present in a cell in order to change the indicator color. It is therefore impossible to determine the positions of the enzyme in the cells by the indicator method unless the urease molecules are sharply localized in specific regions.

Summary

1. Two methods for the determination of urease are described. The first, a histological method, depends on detecting the increase in alkalinity of the cells as urea is being hydrolyzed by the urease present in the cells. This increase in alkalinity is made evident either by the use of a suitable pH indicator or by the formation of a lake. The second, a quantitative method, depends on the determination of the ammonia produced when urea is hydrolyzed by the enzyme.

2. The following factors were considered in applying the histological method: The hydrogen-ion concentration of the tissue; the suitability of various indicators; the permeability of cells to indicator dyes and to urea; the buffer capacities of cells and reagents; the time factor for the method; and the preparation of tissue sections for analysis. In the lake method, reagents were chosen which would form insoluble and highly colored precipitates at the enzyme points.

[3] Assumptions: Dimensions of a parenchyma cell $20 \times 50 \times 50\ \mu$; density of cell 1.0; there is one active group per urease molecule; urease has a molecular weight of 150,000; 1 gm. of crystalline urease can produce 26,000 mg. NH_3 per minute, which is the highest activity reported by SUMNER (23).

3. The following factors were considered in applying the quantitative method to various tissues: Effect of drying the tissues; effect of macerating the tissues; effect of various urea concentrations on the maximum urease activity; effect of the temperature; effect of the time; the rate of inactivation of urease; the effect of the hydrogen-ion concentration; the choice and concentration of the buffer; the recovery of ammonia by aeration; and the sampling of tissues for analysis.

4. A concept of urease activity, based on the number of available active groups of urease, is discussed.

The writer wishes to express his gratitude for the many helpful suggestions and the kindly criticisms of Professor F. G. GUSTAFSON throughout the course of this investigation.

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A RECORDING POTENTIOMETER FOR USE IN PHYSIOLOGICAL INVESTIGATIONS

R. H. WALLACE

(WITH SEVEN FIGURES)

Introduction

Some years ago during the process of developing a vacuum tube voltmeter it was found necessary to build a recorder that was adequate to test and check the voltmeter. The instrument described in this paper is one evolved from the early recorder during more than three years of experimentation.[1]

It was found upon investigating the many types of recorders on the market that none could be said to be adaptable enough or to have the numerous other characteristics required for use in physiological problems. For this reason the fundamental ideal kept constantly in mind during the development of this instrument was to make every part as universal and as adaptable as was possible without sacrificing dependability or accuracy.

Characteristics of the instrument

SENSITIVITY

Perhaps the first characteristic that we demand of an instrument is sensitivity. In this respect the one described below leaves little to be desired. As ordinarily adjusted the present instrument is activated by 0.1 microampere or, in terms of voltage, 0.1 millivolt. If, however, higher or lower sensitivity than this is desired it can be obtained. With maximum adjustment for sensitivity the instrument can be made to work well on 0.01 microampere. If, on the other hand, the potentials being measured are high, one can, by suitable shunts across the null galvanometer, tone the instrument down so that even tenths of volts are necessary to activate it.

MILLIVOLTAGE RANGE OF BRIDGE

The one characteristic that is more inadequate than any other in most commercial recorders is the failure to equip them with a universal bridge. Only one or at most a few different scale values are given, and anything that is being measured which does not fit these arbitrary units must be modified by shunting or other devices to bring them within the scale of the instrument. In the present case this is entirely eliminated. The bucking voltage of the instrument is furnished by dry cells. If the potential which we are to measure does not exceed 1500 millivolts, ordinary flashlight dry

[1] The writer wishes to express his appreciation to JOHN L. REINARTZ for demonstrating the feasibility of this type of instrument.

cells are used, but if greater values than this are needed one can substitute the number of 45-volt "B" batteries needed.

Also by means of a suitable arrangement of resistances one can adjust the bridge so as to get any millivoltage from 3 to the full value of the battery. This is done by the mere turn of the required dial. In addition to this universal control of the millivoltage value of the bridge, one can likewise place the zero point of the bridge at any desired location between the two ends of the bridge. But even more important than this is the fact that the shifting of the zero for any point between the two ends of the bridge can be done without modifying the sensitivity for which the bridge is set. Below will be found the method whereby these adjustments are accomplished.

Calibration

Calibration is made with the usual potentiometer. One simply decides the millivoltage in which he is interested and then sets up a potentiometer with this value. He then connects this to the imput leads of the recorder and adjusts the sensitivity control resistance so that this millivoltage gives him the centimeter deflection on the recorder drum that he desires. He then adjusts the zero control dial so that the zero is where he wishes it. Further information on this point will be found below. Under ordinary conditions this calibration is good for months. If at any time the batteries begin to fail, the action of the instrument makes this immediately obvious. Ordinarily the batteries have shelf life; that is, they last just as long as if they were stored on the shelf—at least one year is reasonable. Obviously one can recheck the calibration in a few minutes at any time.

Stability

The instrument has functioned for months with no attention other than the replacement of the pilot bulb once in a while and other minor items of that sort. No fundamental element of the instrument has been replaced in a year of continuous use. The instrument even functions well when line voltage fluctuates from 100 to 120 volts. On one occasion when the instrument was being used as a light recorder illness prevented me from being in the laboratory for a period of two weeks. Nevertheless, when I did return, there were the complete records for the period.

Length of record and speed of movement of paper

The drum can be made to turn once a minute, once an hour, or twice a day, depending upon the shaft of the clock that is used for attachment. For most purposes however it is set to turn twice a day. Since the drum itself is 36 cm. in circumference, the speed of movement of the paper is 3 cm. per hour. A 150-foot roll of recording paper can be put in at one

time, which is a month's supply (day and night run) when the drum revolves twice daily.

Availability and cost of the instrument

The instrument can be built by anyone handy with his hands and able to do simple wiring. A complete set[2] of five blueprints is available showing the full size and shape of all parts and the exact connections to be made. Two types of wiring schemes are given. One type, the ordinary schematic one of the radio engineer (fig. 2), that shows grids, cathodes, plates, etc., means something to those initiated into the realm of the radio but it means little to the rest of us. The other wiring scheme is purely pictorial and shows the exact size, spacing, shape, and connection for all parts (figs. 4, 5). The total cost, if one purchases all the mechanical parts ready-made but does the wiring himself, is about $125. This price, of course, includes all educational discounts. Anyone who understands the radio circuit type of wiring should complete that part in a day. The adjustment may require somewhat longer.

How the instrument works

Fundamentally this instrument is a self-balancing Wheatstone bridge of the null-indicator type. That is, a voltage is put across the two ends of a resistance wire. If one connects low resistance wire to one end of this system and then touches the other end of the wire to any point along the resistance wire, a meter in series with the low resistance wire will deflect, showing that there is a voltage along the first wire. This voltage constitutes the bucking voltage against which the unknown, which is being measured, acts. The rest of the instrument consists merely of the various elements necessary to move the traveling arm back and forth as the voltage of the unknown changes so that at all times the voltage on the resistance wire balances to 0.1 millivolt the voltage of the unknown. When this condition is attained the instrument is in balance and the meter in series with the low resistance wire shows no deflection; that is, it is null.

The bridge

Let us now refer to figure 1A to see how the universal bridge works. Assume that the wire AB is the bridge of the recorder and that R is the potentiometer controlling the sensitivity of the bridge. Then if U is a 1.5-volt (1500 mv.) dry cell and R is turned to no resistance, that is, t is up

[2] This complete set together with list of parts needed, catalog numbers, addresses of places of purchase, and all other information for the construction and adjusting of the instrument can be obtained for $1.00 by writing to R. H. Wallace, Storrs, Conn. All figures used in this paper as well as the originals from which the blueprints are made were drawn by H. E. Hill.

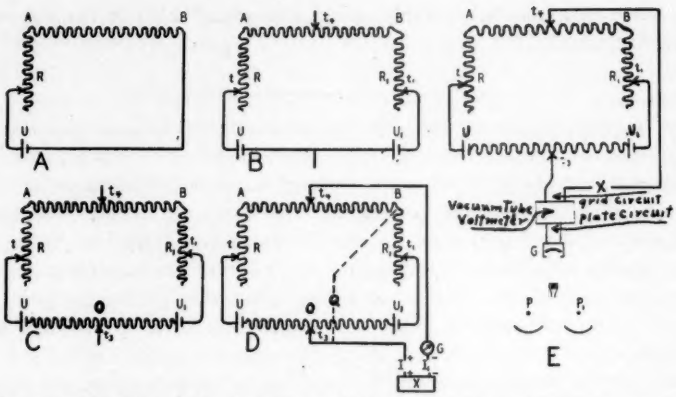

FIG. 1. Schematic wiring diagrams of bridge and method of connecting the bridge to a vacuum tube voltmeter. A, basic diagram of circuit showing how the voltage on bridge *AB* can be controlled by the adjustment of the variable resistance *R*. B, circuit similar to A, except resistance R_1 and battery U_1 have been added making circuit balanced with zero between *A* and *B*. C, same circuit as in A and B with resistance *O* added to control the position of zero without modifying the sensitivity of the bridge. D, complete schematic diagram of the bridge of the recorder showing the method of connecting the null-control galvanometer and the unknown *X*. E, necessary modification of the bridge connections when the recorder is used with a vacuum tube voltmeter. For details regarding the bridge circuit and how it works see text.

to point *A*, then the full voltage of *U* or 1500 millivolts will be across the bridge from *A* to *B*. The resistance of wire *AB* is 500 ohms and that of *R* is 25,000 ohms, so that the ratio of the two is 1 : 51. If then *R* is turned to full resistance (25,000 ohms), the voltage between *A* and *B* will divide up now in the ratio of this resistance or 1 to 51 so that the voltage now present from *A* to *B* will be 1500 ÷ 51 or 29.4 millivolts. With this combination the bridge can be set for any value between 29.4 and 1500 millivolts, depending upon how much is put in from resistance *R*. Automatically, however, zero voltage is at *B*, since there is no resistance between *B* and the terminal of the battery. This zero point cannot be shifted.

Suppose (fig. 1B) we now add another resistance (R_1) which is identical to *R*. With *R* and R_1 now set for no resistance the voltage across *AB* becomes double that above because U_1 has been added and it is now 3000 millivolts. On the other hand, if both *R* and R_1 are set for full resistance, the voltage across *AB* will divide as 1 is to 101, since the ratio of resistance is now 500 : 50,500. The total voltage between *A* and *B* will therefore be 29.7 millivolts. Now, however, the zero point has shifted up from *B* to some point near the middle of the bridge so that point *A* is 14.85 millivolts plus with respect to it while point *B* is 14.85 millivolts minus to it. The

zero point can be shifted back and forth by changing the resistance value of R or R_1, but one will be modifying at the same time the sensitivity of the bridge.

Suppose we now add a third resistance (O, fig. 1C), which is equal to or slightly greater than the resistance of AB. If this resistance is set with the traveling arm exactly in the middle of its resistance wire then the zero point on AB will be unchanged. If it is moved toward U, however the zero on the bridge will automatically shift an equal distance toward B; while if it is moved toward U_1, the zero will move toward A. Since the resistance of O is equal to or greater than that of AB, therefore the zero can be shifted to any point between A and B. But notice this also; as you add more resistance to the U-side of the resistance O you take it away from the U_1-side. Consequently, the total resistance in the circuit between the traveling arm t_4 of the recorder bridge and t_3 of the zero adjusting potentiometer remains exactly the same. The zero can be shifted but the sensitivity is unchanged.

Reference to the dotted line in figure 1D will show another very valuable addition to the universality of the bridge. If one is measuring something which can drop to zero voltage, but never change sign, then, one can close switch Q and use the new bridge combination. When Q is closed, battery U_1 and resistance R_1 are shorted off the bridge AB. They are shorted on themselves, but this does no harm since resistance R_1 is 25,000 ohms and therefore insures shelf life for battery U_1. The bridge has now become a simple one like that in figure 1A, except that resistance O is in the circuit. The attachment of the wire from Q to the moveable arm t_3, rather than directly to battery U, enables one to set zero for any point between the two ends of the bridge the same as in figure 1B above. This setting of zero entails modification of the sensitivity of the bridge, however, so that one must obtain the combination of zero and sensitivity of bridge by adjusting both R and O. The value of this zero adjustment will be discussed below.

Another valuable characteristic of this new bridge combination is the increased sensitivity of the null point which it gives. Thus, if one examines figure 1D he will see that the circuit from the unknown (X) must be completed through the zero-adjusting arm t_3 and the traveling-arm t_4 of the bridge. Between these two points we have R and R_1 in parallel which means that they act as one resistance of 12,500 in series. Also in series with this 12,500 ohms will be one-half of the total resistance (assuming t_3 and t_4 are on center) of bridge AB and resistance O, since they likewise are in parallel. We may then have as much as 13,000 ohms in series with the galvanometer when Q is open. On the other hand, when Q is closed the maximum is 1000 ohms. Since the null-control galvanometer itself has 1000 ohms resistance, this additional 1000 ohms will reduce its deflection per unit of voltage to just one-half when Q is closed. When Q is open, on the other

hand, it will be reduced to 1/13. The instrument has so much sensitivity that even this loss is not important for most measurements, but in some cases this additional sensitivity is quite valuable.

Figure 1E shows the slight modifications in the wiring when the recorder is to be used with a vacuum tube voltmeter. The null-control galvanometer is disconnected from the bridge circuit and hooked directly in parallel with the plate circuit of the vacuum tube voltmeter. The unknown remains connected as in figure 1D, and the grid circuit of the voltmeter is connected directly in series with it.

We are now ready to complete the null connections (fig. 1D). If we now connect an unknown (X) to the imputs I and I_1, so that polarity bucks that from the bridge, the galvanometer (G) will deflect. If some suitable mechanism is activated by the deflection of G which can cause traveling arm t_4 to move, then more or less voltage will be put into the circuit, depending upon which is required. When the voltage from the bridge exactly equals that from the unknown, then G will no longer deflect and the mechanism will be in the null condition. This mechanism for maintaining null constitutes most of the remaining story of the apparatus.

Null-activating device

Electrical arrangements.—Figure 2 gives the schematic writing diagram of the entire mechanism that maintains the galvanometer on null. The galvanometer (G) is placed directly in front of the pilot bulb (F_1) and also in front of the two symmetrically placed photoelectric cells (P and P_1). These photocells are attached to the control grids of the two mercury vapor

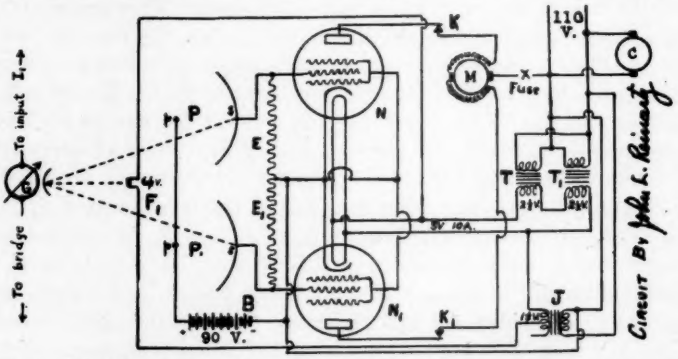

Fig. 2. Schematic wiring diagram showing all electrical connections necessary in the automatically maintained null. G, null-control galvanometer with mirror; F_1, pilot bulb; P, P_1, photo-electric cells; p, s, pin and shield of photo-cells; E, E_1, grid leaks; N, N_1, mercury vapor tubes; K, K_1, limit switches; M, reversible motor; X, fuse; T, T_1, and J, transformers; C, electric clock to turn drum. For further details see text.

tubes (N and N_1) with grid leaks (E and E_1) suitable for these tubes. The plates of these mercury vapor tubes are connected through the limit switches (K and K_1) to the split fields of the reversible motor (M). The twin transformers (T and T_1) supply the 5-volt 10-ampere filament supply to the mercury vapor tubes. The supplementary transformer (J) is connected in series with these twin transformers to increase the voltage to $6\frac{1}{4}$ volts to supply the pilot bulb (F_1). C represents the electric clock that drives the recording drum.

When the unknown is exactly balanced by the voltage on the bridge, the light from the pilot bulb (F_1) hits the mirror of the galvanometer (G) and is reflected back between the photocells (P and P_1), the mercury vapor tubes are not activated, and the motor carrying the travelling arm on the bridge does not run. When the circuit is unbalanced, however, then the light may reflect back and hit the photocell (P). This causes the shield (s) to become plus and the voltage from the batteries (B) is allowed to pass and put a plus charge on the control grid of the mercury-vapor tube (N). This in turn allows the current to flow through the tube from the 110-volt circuit to one field of the motor (M), causing the motor to run in one direction. If, on the other hand, the light is reflected on the tube (P_1), the same series of events occur for that side of the circuit and the motor runs in the reverse direction. The only connection between the bridge circuit and this activating mechanism circuit is the beam of light reflected back from the galvanometer mirror. In the early models of this recorder relays were used instead of the mercury-vapor tubes, but since they continually gave trouble they were eliminated.

The radio tube in its simplest form consists essentially of a cathode, a grid, and a plate. This tube is connected into a circuit in such a manner that current tends to flow from the cathode, which is minus, to the plate, which is plus. This flow can take place only when the grid, which is between the cathode and the plate, is likewise plus. A radio tube can therefore be said to be an electronic relay. Most tubes allow only a few milliamperes of current to pass. The mercury vapor tube varies from these in being able to let many amperes through. The type used in this circuit will pass 4.5 amperes at 1000 volts. This gives a current-carrying capacity many times that required for the 1/80 H. P. motor used in this instrument.

Reference to figures 2 and 5 will show limit switches (K and K_1). These are put in the plate leads from the mercury-vapor tubes and the fields of the motor to cut the motor out in case the traveling arm tries to overrun at the limit of its run. This eliminates any possibility of the instrument being damaged. Likewise it will be noted that at X there is a fuse in the common lead to the fields of the motor. This is to prevent damage in case both sides of the circuit are activated at the same time and the motor tries

to go in both directions at once. It will be noted that the clock for driving the drum is connected in directly in parallel on the 110-volt line. In figures 4 and 5 are given the self-explanatory pictorial diagrams of the spacing of parts and wire connections.

OPTICAL ARRANGEMENTS.—Figure 3 gives a section through the instrument showing the exact vertical spacial relationship between the various

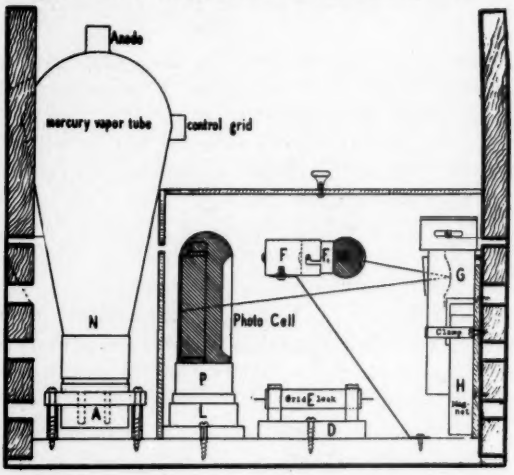

FIG. 3. Section through instrument showing exact spacial relationship of all parts. In this figure, and those following, these parts are shown in their true shapes and relative sizes.

parts. These relations must be followed very closely to obtain a workable optical arrangement. The shading on the pilot bulb (F_1) and the photocell represent photographer's opaque which is used to mask out the excess light. Figure 4 shows the exact horizontal spacial relationship of all these parts.

MECHANICAL ARRANGEMENTS.—Figure 6 shows the motor with its reducing gear, the screw rod carrying the traveling arm, and the spacing and mode of attachment of the drum. It will be noted that opposite the traveling arm of the bridge there is another arm which moves along with it that is at the same time tracing the record on the drum. The recording paper is a wax-covered paper which requires no ink but merely pressure from a writing point to give a record. Figure 7 shows the spacing of parts on the front panel of the instrument. This spacing must be followed closely to enable one to get in all the parts as shown. The roll of paper is here shown as a full-sized roll of 150 feet.

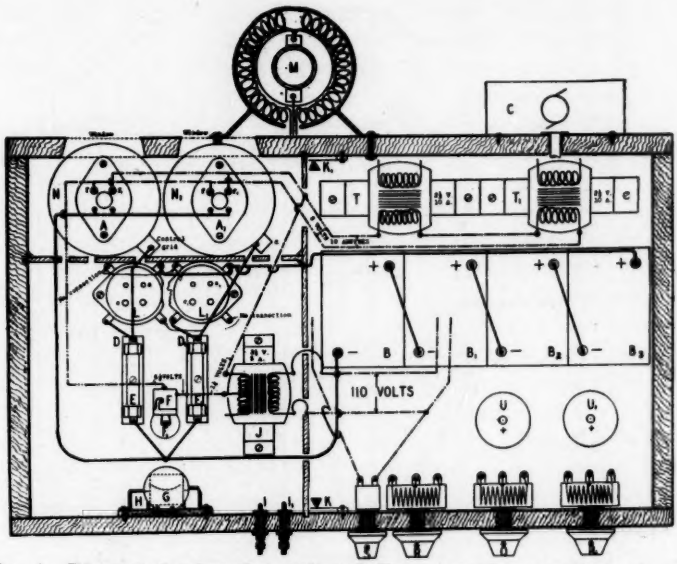

FIG. 4. Diagram showing photo-unit and filament-supply connections of the null-maintaining mechanism.

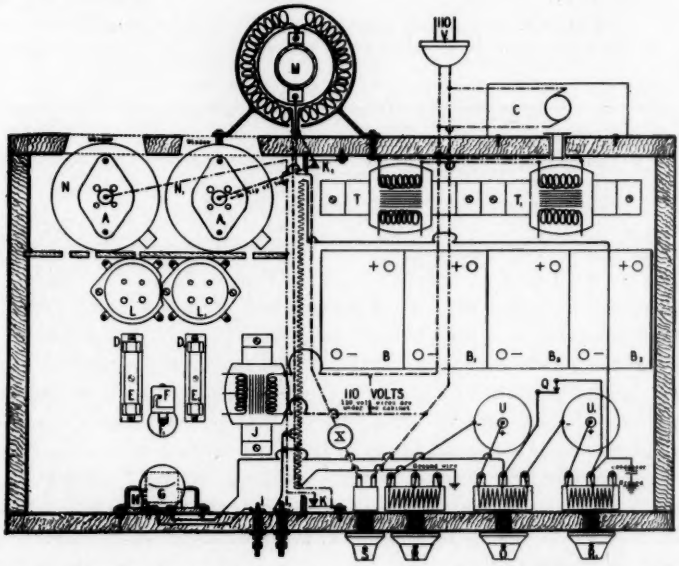

FIG. 5. Diagram showing the 110-volt connections of the null-maintaining mechanism and the wiring of the bridge.

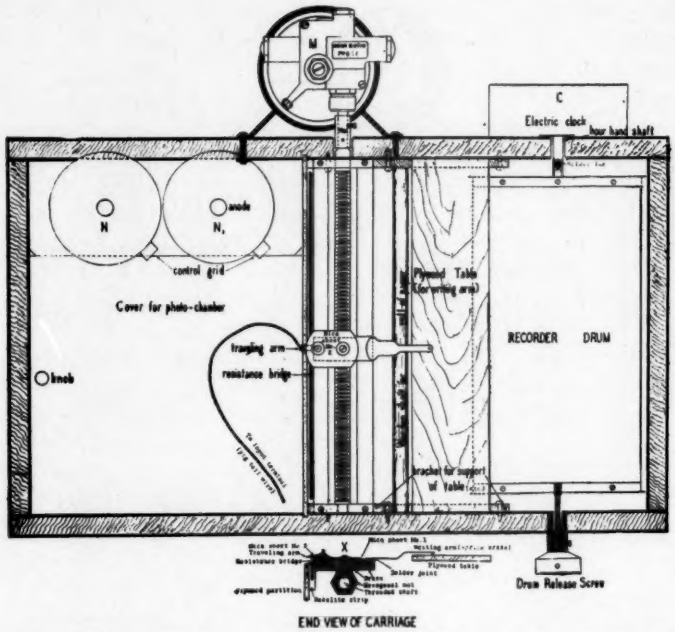

FIG. 6. Diagram showing the spacial arrangements of the motor, traveling arm of bridge, writing arm, screw drive, drum, drum release, electric clock, etc., as seen from above.

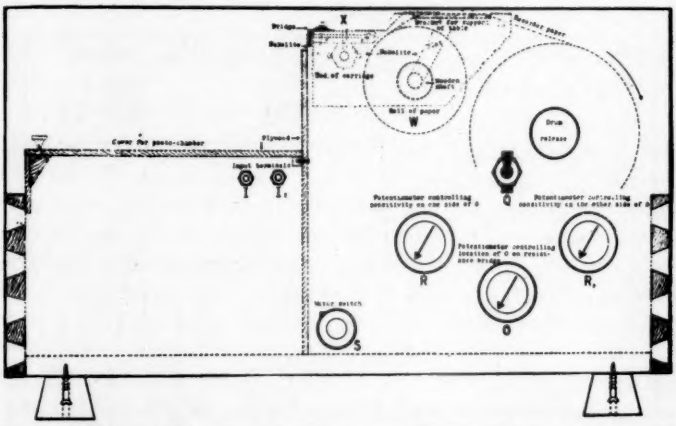

FIG. 7. Diagram showing both horizontal and vertical spacing of all parts in the front panel of the instrument.

The writer will not attempt to give in this paper the numerous details of construction and manipulation of the recorder. These will be found, however, in full detail on the blueprints or in the supplementary information enclosed with them. It may be said, however, that while this additional information greatly simplifies the work, nevertheless, anyone familiar with amateur radio construction can with no difficulty construct an instrument from the information here given.

A double recorder can likewise be constructed from these parts with only very minor modifications. This double recorder is made of two entirely separate but identical bridges and null-activating mechanisms inclosed in one cabinet and writing on one drum. The ground plan of this instrument is simply the plan as shown in figures 4 and 5, with the mirror image of this plan used on the other side of the drum. This instrument has the same fundamental characteristics as the single recorder. The bridge arrangement controlled by the switch (Q) gives one complete control of the location. of zero. This is a very valuable characteristic to have in a duplex recorder. Thus one can set the two unknowns under test for equal centimeter deflection on the drum with their zeros on the same base line. Furthermore, one zero can be 1 cm. below the other, or there can be any other combination desired. One can even have one base line at one end of the drum and the other at the other end and have the curves move toward each other, or have them in the center of the drum and measure in opposite directions. Each of these combinations has its special suitability for certain materials under measure. The quality of work which this instrument can do will be amply shown in a study of light sampling to appear in an early issue of this journal.

Application of the instrument

Recording potentiometer or millivoltmeter

This instrument can be used in experiments involving a great number of physiological problems because of the adaptability of the various parts. It has worked excellently for testing the stability of vacuum tube voltmeters and for recording voltages being measured by means of a vacuum tube voltmeter. It has also been used on hydrogen ion and electrometric titration. The chief application so far made of it has been the recording and study of methods of measuring light. It is peculiarly adapted to this use because of its universality. Some preliminary tests have been made of it with thermocouples for temperature measurements, and it seems equally well suited for this work. A study of the characteristics of the instrument will indicate to anyone many other applications.

Automatically maintained null

A very valuable application of this instrument is that of an automatically maintained null device accurate to 0.1 microampere or 0.1 millivolt. That is, one can use it to measure pH or to maintain pH. If one sets the traveling arm of the bridge for some pH he desires and then disconnects the anode leads of the two mercury vapor tubes from the motor and connects them instead to an acid- and an alkali-adding device, the pH value can then be maintained with very great accuracy. The mercury vapor tubes are capable of running an $\frac{1}{3}$-horse power motor which gives some idea of the power available for maintaining any type of null desired.

The writer does not consider the present instrument perfect, and therefore welcomes all criticisms and suggestions for its improvement. In this manner, although the instrument may be far from perfect at present, we can hope finally to develop an instrument ideally suited to the very exacting requirements of physiological work. This instrument had its inception in the study of vacuum tube voltmeter stability. It is not and, in the very nature of the many problems involved in its evolution, cannot be the work of one person. It is therefore with the very greatest pleasure that I express my indebtedness to E. L. Kelly, D. G. Steele, John L. Reinartz and L. B. Crandall for their invaluable assistance. That which this instrument is, it is through the cooperative efforts of us all.

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CONTRIBUTION TO THE KNOWLEDGE OF PLANT PHENOLASES[1]

RUDOLF M. SAMISCH

(WITH ONE FIGURE)

Introduction

Respiratory chromogens are thought by PALLADIN and his coworkers (**16, 17**) to play an important rôle in plant respiration. Dihydroxy and trihydroxy phenols are found among these chromogens. They are oxidized to quinones by molecular oxygen in alkaline solution. In acid solution, a similar oxidation occurs in the presence of oxygen and plant phenolases. The possible rôle of phenolase in plant respiration has been questioned, because attempts to correlate activity with rate of respiration have failed; furthermore, phenolases could not be found in a large number of plants. It should, however, be born in mind that a correlation between phenolase activity and rate of respiration can be expected only if the enzyme is the limiting factor in the system. That this is not the case can be seen from the fact that the addition of phenolic compounds to a plant extract known to contain phenolase is followed by the development of colored products. In other words, the amount of enzyme present is larger than that required for the oxidation of a plant chromogen normally present and the chromogen appears to be the limiting factor. The fact that phenolases have not so far been demonstrated in a number of plants may be due to the fact that the possibility of specificity among phenolases such as is known to occur among other enzymes has been ignored. Thus the reagents used for the determination of their presence may have contained reactive groupings that are attacked by some phenolases but not by others. That such a condition prevails will be shown in this paper, in which observations are reported which appear to indicate the existence of three phenolases, specific for the ortho-, meta- and para-hydroxy grouping respectively.

Methods

The plant extracts were prepared from frozen material by extraction with 1 per cent. sodium bicarbonate solution. The extracts were immediately neutralized with glacial acetic acid and saturated with toluol. Phenolase activity was measured by observing manometrically the rate of oxygen absorption by the extract-substrate mixture in a Barcroft-Warburg respirometer. The reaction took place under oxygen at 25° C. in a

[1] From the Division of Fruit Products and the Division of Plant Nutrition, University of California, Berkeley.

medium buffered with an acetate mixture. None of the extracts showed a measurable oxygen absorption in the absence of a suitable poly-phenol. The details of the method have been discussed elsewhere (**19**).

Experimentation

The catalyzing power of different plant extracts was tested on various phenolic substrates at varying concentrations and pH. The extracts from fruits of apricot (var. Royal) and avocado (var. Spinx) oxidized catechol very rapidly and pyrogallol only slowly. The ratio for avocado was about 6:1[2]. Avocado extract oxidized phloroglucinol at an exceedingly slow rate, while apricot extract caused observable effect. Neither avocado nor apricot extracts oxidized phenol, resorcinol, quinol, or tryosine, although potato extracts oxidized tryosine at a rapid rate. The avocado and apricot extracts were used with resorcinol between 0.1 and 1 M concentration from pH 3.9 to 7.1, and with quinol at 0.15 and 0.3 M concentration between pH 3.9 and 7.1. The tyrosine was used only at pH 6.5.

An extract of lemon leaves (var. Eureka) was found to oxidize phloroglucinol very rapidly, but it oxidized resorcinol very slowly (pH 6.2, conc. 0.5 M). Catechol and pyrogallol were oxidized at a considerably slower rate than phloroglucinol (table I). No oxidation of phenol or tyrosine was obtained. The ratio of the rate of phloroglucinol oxidation to that of catechol was about 11:1. A rather interesting phenomenon observed was the action of catechol upon the phloroglucinol-oxidase system. Catechol inhibited the oxidation of phloroglucinol (table I).

TABLE I

EFFECT OF CATECHOL UPON THE RATE OF OXYGEN ABSORPTION BY LEMON LEAF EXTRACT IN THE PRESENCE OF PHLOROGLUCINOL AT pH 6.2 AFTER 20 MINUTES (IN MG. × 10^3)

SUBSTRATE	0.0125 M PHLOROGLUCINOL	0.0125 M PHLOROGLUCINOL 0.05 M CATECHOL	0.05 M CATECHOL
Enzyme and substrate	315	166	138
Substrate blank	0	90	110
Enzyme and substrate corrected for blank	315	76	28

After exposure to oxygen without the enzyme the phloroglucinol remained almost colorless although having a greenish-yellow tinge; the catechol had browned; but a mixture of the two became green. When the enzyme was

[2] Unless stated otherwise, this ratio refers to absorption of oxygen under optimum substrate concentration.

present the mixture of the two substrates turned greenish black. The mixture of catechol and phloroglucinol prepared in a test-tube prior to the determination remained almost colorless in absence of the buffer. When auto-oxidation was hastened by raising the pH the dark green pigment developed. The amounts of absorption by the substrate blanks (table I) seem to indicate that the presence of phloroglucinol only slightly inhibited the auto-oxidation of catechol. From this we may infer that the reactive OH groups of the catechol were not involved in the reaction causing the formation of the green pigment associated with the inhibition of enzyme action of phloroglucinol in the presence of catechol. A possible explanation of the inhibition of phloroglucinol oxidation by catechol would seem to lie in a reaction of the phloroglucinol with the oxidation product of the catechol.

The extracts from young pear leaf (var. Bartlett) very rapidly oxidized quinol. Catechol was oxidized at about one-fourth the rate of quinol (table II), and pyrogallol still more slowly. Resorcinol, phloroglucinol, phenol, and tyrosine were not oxidized to a measurable extent.

The various extracts had different properties in respect to their power of oxidizing different phenolic groupings. Apricot and avocado extracts oxidize most rapidly the ortho-hydroxy-phenol, lemon extract the meta-hydroxy-grouping, and pear the para-compounds. More than one such group was attacked by most extracts. On the assumption that more than one enzyme was present, attempts were made to separate them. The enzymes were fractionally precipitated by alcohol, filtered, dried, and redissolved in water. The relative activity of the precipitate for different substrates was tested. However, no significant differences could be obtained because of the great differences in the rate of catalysis of any one plant extract toward any two substrates. This necessitated correspondingly large dilution of the stronger one, thus increasing the error considerably. The enzymes in avocado, apricot, and lemon could be precipitated with 75 per cent. alcohol while the enzymes in the pear could even be precipitated with 45 per cent. alcohol.

It has been shown (**21**) that the catalytic power of apricot extract is destroyed by exposure for only 1 hour at 70° C. Extracts of pear leaf and lemon leaf (without buffer) were brought to boiling and then immersed in boiling water for one-half hour. Table II shows the effect produced by boiling. While the power to oxidize catechol was lost by the lemon leaf extract, it was still able to slowly oxidize phloroglucinol. The power to catalyze the oxidation of quinol had been completely retained by the pear leaf extract while it had lost most of its action on catechol. Thus in the same extract one catalytic power could be destroyed, while the other remained unharmed, which lends strong support to the theory that we are dealing with more than one enzyme.

TABLE II

EFFECT UPON PHENOLASE ACTIVITY OF HEATING FOR ONE-HALF HOUR AT 100° C.

SOURCE OF EXTRACT	SUBSTRATE	pH*	$O_2 \times 10^3$ MG. ABSORBED† AFTER 10 MINUTES	
			UNHEATED	HEATED
Lemon	0.5 M catechol	5.6	97	0
	0.0125 M phloroglucinol	6.3	2670	47
Pear	0.5 M catechol	5.6	56	8
	0.05 M quinol	6.0	236	237

* pH at which oxygen absorption was determined (buffer added after heat axposure).
† All values corrected for auto-oxidation.

These extracts were purified by precipitation and reprecipitation with three times their volume of alcohol, and their pH-activity curves (fig. 1) and Michaelis constant were determined. The activities, as influenced by pH, are plotted here on different scales in order to be able to show the curves on one graph. In each case, however, the intersection of the ordinate on the abscissa is used as the zero point. While the apricot-catechol and the lemon-phloroglucinol curves show definite optima in the acid range, no optima occur in the pH range studied for the pear-quinol and the avocado-pyrogallol systems. No measurements were taken above pH 6.2 because of the greatly increased auto-oxidation of the substrate. It has been pointed out (**20**) that the catechol system, when obtained from different sources, shows an optimum pH, which is presumably due to the accompanying specific colloids as it differs for different plants. Further work would probably establish a similar relative pH relationship between the various enzyme-substrate systems in different plants.

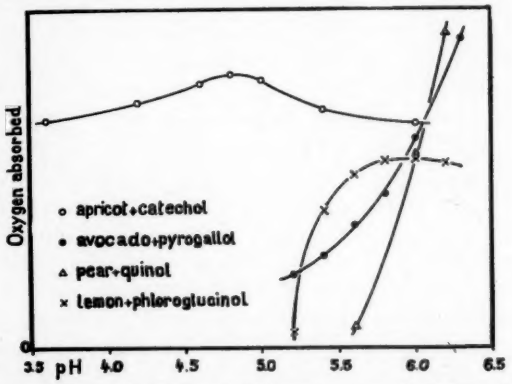

FIG. 1. pH-activity curves of different enzyme-substrate systems.

The Michaelis constant was determined by plotting substrate-concentration-activity curves and finding by this graphic method the concentration at which 50 per cent. activity was obtained. The results are shown in table IV.

TABLE IV

MICHAELIS CONSTANT OF DIFFERENT PHENOLASES

SUBSTRATE	CATECHOL	PYROGALLOL	PHLOROGLUCINOL	QUINOL
Source of extract	Avocado Apricot	Avocado	Lemon	Pear
Michaelis constant	7×10^{-2}	$2\text{–}3 \times 10^{-2}$	6×10^{-3}	$1\text{–}2 \times 10^{-2}$

These values, ranging between 6×10^{-3} and 1×10^{-2}, differ in their order of magnitude from those reported in the literature. Thus WARBURG (**24**) found oxygenase of yeast to have a K_M of 0.5×10^{-7} M oxygen, and WILLSTÄTTER and WEBER (**26**) reported a $K_M = 6 \times 10^{-6}$ M leucomalachite green for peroxidase of horse-radish. These and similar values for other oxidases caused HALDANE (**8**) to suggest that the low constant of oxidases may prove to be a fundamental distinction from hydrolytic enzymes. EULER and LAURIN (**5**) have shown that purification does not influence the Michaelis constant. The different values which they obtained for the Michaelis constant of saccharase from various races of yeast seem to be due to the presence of inactive enzymes which have retained their affinity for the substrate but do not dissociate from it. Since this would tend to produce lower values rather than higher ones, this factor does not seem to enter into the divergence which is reported here from the results of other workers.

Discussion

The observations reported in this paper tend to indicate that phenolases from different sources may be of a different nature. This expresses itself primarily in their specificity towards definite groupings. BERTRAND (**1**) compared the relative rate of oxidation of phenols in the presence of lactase and found the rate of oxidation was in the order: quinol > catechol > resorcinol. More recently BUNZEL (**2**), working with various organs of the potato plant, came to the conclusion that the order of oxidation by oxidase was: para > ortho > meta. On the other hand, he found later (**3**) that this relation may not hold true in other plants and that the relative order of ease of oxidation of different phenols may vary in different plants. In no case was the meta position found to be easily oxidized.

The writer's plant material has the advantage that in all cases tyrosinase was absent, and that it was not measurable by the test-methods employed. This enzyme, which OKUYAMA (**14**) has shown to be essentially

a dehydrogenase, may be responsible for the high rate of oxidation of the para grouping reported in some of the data. In each of the three types of material under discussion a di- or trihydroxy phenol with a different relative position of the hydroxy groups in the phenol ring was preferentially oxidized by the enzyme. We prefer to speak of relative rates rather than of absence of the oxidation of certain groupings because the oxidation may have taken place at such a slow rate that it remained unnoticed under the conditions of the experiment.

It is, however, of interest to note in connection with the predominant rôle which ONSLOW (**15**) places upon catechol compounds, that the ortho grouping was more or less oxidized by the extracts in all three cases. Furthermore the oxidation of pyrogallol was slower than that of catechol, which shows that a group in a different position than those directly attacked may influence the reaction.

These findings give rise to the following question: Do the preparations represent different enzymes, or is there fundamentally one enzyme with its activity modified by various plant colloids? BUNZEL (**3**), who did not have quite as striking a case with his material, thought that the modification in the relative rate of oxidation of several substrates by various plants was due only to differences in the colloidal substances accompanying the enzyme. He stated that this contention was supported by the fact that all organs of the same plant behaved alike, while if he had dealt with a mixture of enzymes, they would have been expected to differ in their behavior. Yet his data do not seem to bear out his conclusion inasmuch as various organs apparently show such differences.

Of the three types of extracts discussed in this paper, the pear-leaf extract can be readily singled out from the other two. Para-phenolase of pear leaves is heat stable while the accompanying ortho-phenolase is easily destroyed by heat. Indeed, the heat stability of the former would lead one to believe that it is an inorganic catalyst. Heat stable oxidases have been reported in alfalfa (**4**), tobacco (**6**), and mushroom (**25**). Furthermore a major part of this para-phenolase was precipitated by the addition of an equal volume of alcohol to the solution, while the other phenolases necessitated the use of a higher concentration of alcohol for precipitation.

The enzymes of the lemon-leaf extract could not be separated by fractional precipitation with alcohol. Indeed, the fact that catechol greatly retarded the oxidation of phloroglucinol by lemon-leaf extract may provide an explanation for the reported very weak oxidation of the meta grouping found in the case of avocado. Avocado reacts positively to the ferric chloride test used by ONSLOW (**15**) for the identification of catechol compounds. Citrus was classed by her among "peroxidase" plants for the lack of response to this test. This would seem to suggest that we may

be dealing with the same enzyme, the oxidation of the meta grouping in avocado and other "catechol plants" being inhibited by the presence of an ortho-phenol. However, it is difficult to see how such a small concentration of ortho-phenol, as naturally occurs in the plant, should completely inhibit meta oxidation as, *e.g.*, in apricot. While much higher concentrations used *in vitro* for lemon extract fail to stop it completely. The inhibition of meta oxidation by catechol cannot be explained on the basis of a greater combining power of the enzyme with catechol as compared with phloroglucinol because the Michaelis constant of the meta oxidation is only half that of the ortho reaction, showing that the meta grouping is more strongly attached to the enzyme.

Thus we found an oxidase able to oxidize ortho-phenols in apricot and avocado, plants containing tannins which gave a test for the catechol grouping. The concentrated tannin extracts of apricot (**20**) and solutions of commercial tannins were rapidly oxidized by the diluted oxidase of apricot and avocado. Lemon-leaf extract oxidized phloroglucinol preferentially, while in common with other citrus plants it is known to contain hesperidin, a glucoside of a meta-phenol. A para-phenolase was expected in pear leaves, because they are known to contain arbutin, a glucoside of quinol, and it was actually found to be present in large amounts. Apparently different specific oxidases and their proper substrates are present in different plants.

The question need be raised, as to why the oxidation of these phenolic chromogens does not take place in so many plant tissues unless they are injured, in view of the fact that both substrate and enzymes are present in the vacuolar sap (**18**). This failure of pigment formation in the living cell, assuming the amount of the enzyme or any additional oxidative agent and the pH of the cells to be constant, may be due to one or more of the following mechanisms: (1) the rate of oxygen supply, (2) the amount and availability of the substrate present, or (3) the amount of an additional reducing substance present. The work of STEWARD (**21**) seems to indicate that oxidation and respiration of plant cells at cut surfaces may be limited by oxygen supply and it would seem that the influx of oxygen may play a major rôle in the darkening of cells beneath the cut surfaces of solid tissues. The fact that the substrate may be the limiting factor has been illustrated recently by KERTESZ (**11**). He described the oxidase system of the "Sunbeam" peach, and he believed that the enzyme is apparently not different from that of other stone fruits, but that tannin is almost absent. This variety of peach will not discolor upon injury. In addition to a difference in quantity of the substrate there may also be a modification of its availability. Thus LLOYD (**12**) reports that for various plants tannin may be held at different stages of growth within a gel of cellulose-like substance which may prevent its color reaction with iron salts. A complex

formation of hesperidin, in which the glucoside is surrounded by sugar molecules, has been mentioned by HALL (**10**). These colloidal formations may very well form systems which modify the rate of oxidation in the plant cell by limiting the available free substrate. The presence and activity of hydrolytic enzymes forms a very important mechanism to make the active grouping available for the oxidative system if it is bound, *e.g.*, as glucoside.

A third factor to be considered is the presence of a reducing substance providing for the reversibility of the system. SZENT-GYÖRGYI (**22**) showed that in the potato ascorbic acid could play this rôle. The writer observed the effect of ascorbic acid upon the systems discussed in this paper and found that with all substrates formation of a visible pigment was prevented in the presence of ascorbic acid, while the uptake of oxygen continued. The oxidation of ascorbic acid could be followed in all cases by means of iodine titration. Furthermore, if the pigment had been produced by oxidation of the phenol in alkaline solution, it could be reduced to the leucoform by ascorbic acid. Further evidence of this possible rôle of ascorbic acid in pigment formation is furnished by MORGAN, FIELD, and NICHOLS (**13**), who found that apricots, which had darkened, had lost vitamin C, and by JOSLYN, MARSH, and MORGAN (**10**) who showed that darkening of orange juice would take place only after the amount of ascorbic acid had sunk to a low level. Reducing substances, other than vitamin C, may possibly prevent the formation of the respiratory pigments in plant cells. An enzymatic oxidation of the products obtained by alkaline hydrolysis of sugar has been reported by GUTHRIE (**7**). It would seem probable that the unstable forms of sugar, as they occur in the plant, are able to reduce the quinones, which are formed by the action of oxidases on phenols, or that the oxidized ascorbic acid may, in turn, oxidize other reducing substances as suggested by SZENT-GYÖRGYI (**23**). Such a system may form an important part of the respiratory system in the plant.

Summary

1. The catalytic action of a number of plant extracts upon the oxidation of phenols was tested in a Barcroft-Warburg respirometer.

2. All extracts catalyzed the oxidation of more than one phenol, but preferential oxidation was shown by the various extracts for different phenols.

3. The ortho-phenolase from the fruits of avocado and apricot, which oxidized catechol rapidly and pyrogallol less rapidly, was inactivated by heating at relatively low temperatures. Meta-phenolase from lemon leaves, which oxidized phloroglucinol rapidly and resorcinol exceedingly slowly, was somewhat more heat resistant. Para-phenolase of pear leaf oxidized quinol and was heat stable.

4. The pH-activity curves and the Michaelis constants for these enzyme systems are reported.

5. Attention is called to the fact that glucosides, containing the phenols corresponding to the respective phenolases, are found in the plants examined.

6. The oxidized forms of these phenols could be reduced in each case by means of ascorbic acid, thus showing that the systems are reversible under natural conditions.

The writer wishes to express his indebtedness to Dr. J. P. BENNETT for the valuable aid and suggestive criticism offered throughout this work, to Dr. W. V. CRUESS for his help and the permission to use the facilities of his division, and to Dr. D. M. GREENBERG for valuable suggestions.

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EFFECT OF ULTRAVIOLET RADIATION ON INDOLE-3-N-PROPIONIC ACID

DOROTHY HARE AND H. KERSTEN

(WITH SEVEN FIGURES)

Introduction

Many effects on living as well as on non-living materials have been produced by ultraviolet radiation so that, in view of the recent interest in the group of organic chemicals known as plant hormones, it seemed advisable to the writers to investigate the effect of ultraviolet radiation on one of such substances. Indole-3-n-propionic acid was chosen because it could be obtained easily and because its effect on plants could readily be determined. The work was divided into two parts: one concerned with the effects of the radiation on the acid, and the other with the effects of the irradiated acid on the roots of plants.

Materials and methods

In all of the experiments described, the source of the ultraviolet light was a water-cooled Victor mercury arc operated at 50 volts. Twenty milligrams of the indole-3-n-propionic acid was dissolved in 1 liter of distilled water and irradiated in 500-cc. portions in a quartz cylinder having a Bakelite base attached to the cylinder with Picein. The center of the cylinder, which was 7 cm. in diameter, was placed at a distance of 15 cm. from the arc and the liquid was stirred mechanically during irradiation. Solutions irradiated in small quartz test tubes produced the same effect on plants as those irradiated in the cylinder. Since the change in the acid was not caused by the Picein or the Bakelite the larger container was used throughout.

Experimentation

EFFECT OF ULTRAVIOLET RADIATION ON INDOLE-3-N-PROPIONIC ACID

The following results were obtained from ultraviolet radiation of indole-3-n-propionic acid solutions.

a. Aqueous and alcoholic solutions of the acid are colorless. If they are irradiated for several hours they become yellow. Solutions exposed to the air for the same length of time and otherwise treated in a like manner except for the irradiation remain colorless.

b. Aqueous solutions of the acid are odorless. If they are irradiated for a short period they acquire an unpleasant odor which, after a longer period of irradiation, becomes faintly but distinctly fruitlike. This fruitlike odor

still can be detected in solutions which have been diluted to ten times their original volume.

c. Aqueous solutions of the acid evaporated to dryness at room temperature in a draft of air leave a slightly yellowish residue. Irradiated solutions evaporated to dryness in a similar manner leave a dark brown residue having an unpleasant odor not possessed by the residue from the unirradiated sample.

Alcoholic solutions of the acid evaporated to dryness at room temperature in a draft of air leave a residue of the same color as the acid which was originally dissolved. Solutions of the acid in alcohol, irradiated for eight hours in a quartz test-tube and evaporated to dryness in a similar way, leave a dark brown residue having a disagreeable odor which suggests the presence of indole.

d. Aqueous solutions of the acid reveal a bright blue fluorescence when irradiated with ultraviolet light. The intensity of this blue fluorescence gradually decreases during the irradiation. Its decay can be demonstrated photographically by the apparatus shown in figure 1, in which the light

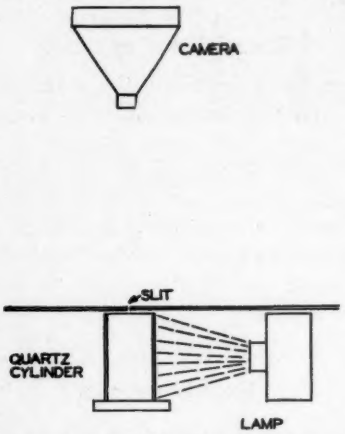

FIG. 1. Diagram of apparatus used to photograph decrease in fluorescence.

coming through a slit placed in the cover of the quartz cylinder is photographed at intervals during the irradiation. In this experiment the procedure was first to photograph the light coming through the slit when the quartz cylinder contained distilled water and then to photograph it at intervals during the time the solution of the acid was being irradiated in the cylinder. Between each photograph the film was shifted slightly so that the images of the slit appeared in adjacent positions on the film. During the intervals between photographs the cover containing the slit was removed

and the solution stirred with an electric stirrer. The result of this experiment was that a progressive decrease in the blackening was observed. This decrease was shown clearly when the relative blackening was measured by a Zeiss microphotometer (fig. 2).

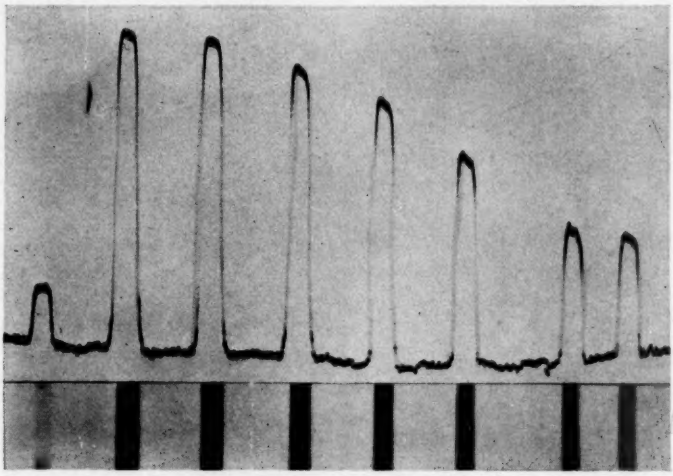

FIG. 2. Below, photograph of the fluorescent light from indole-3-n-propionic acid taken during irradiation with ultraviolet light with the arrangement shown in figure 1. Left to right, water, unirradiated acid, 15 min., 30 min., 1 hour, 2 hours, 3 hours and 4 hours of irradiation. Above, microphotometer photograph, made from the original negative, showing that the fluorescent light gradually disappears with time of exposure.

If a glass which absorbed the visible light but transmitted ultraviolet light was placed between the lamp and the quartz cylinder, the fluorescence appeared as a deeper blue and, although after a period of irradiation the solution remained clear in visible light, it seemed to be milky when lighted by ultraviolet light alone.

e. The fluorescence of the acid solutions extends into the ultraviolet region. This can be demonstrated by replacing the camera shown in figure 1 with a quartz spectrograph. The original spectrum from the ultraviolet lamp appears on the photograph taken in this way, but superimposed upon this is a broad band extending from about 4500 Å to about 3100 Å which gradually disappears as the irradiation progresses. Photographs taken at intervals during irradiation, as well as one taken when the quartz cylinder contained distilled water, are shown in figure 3.

f. Solutions of the acid exhibit a strong absorption band in the ultraviolet region which disappears during irradiation with ultraviolet light. Spectro-

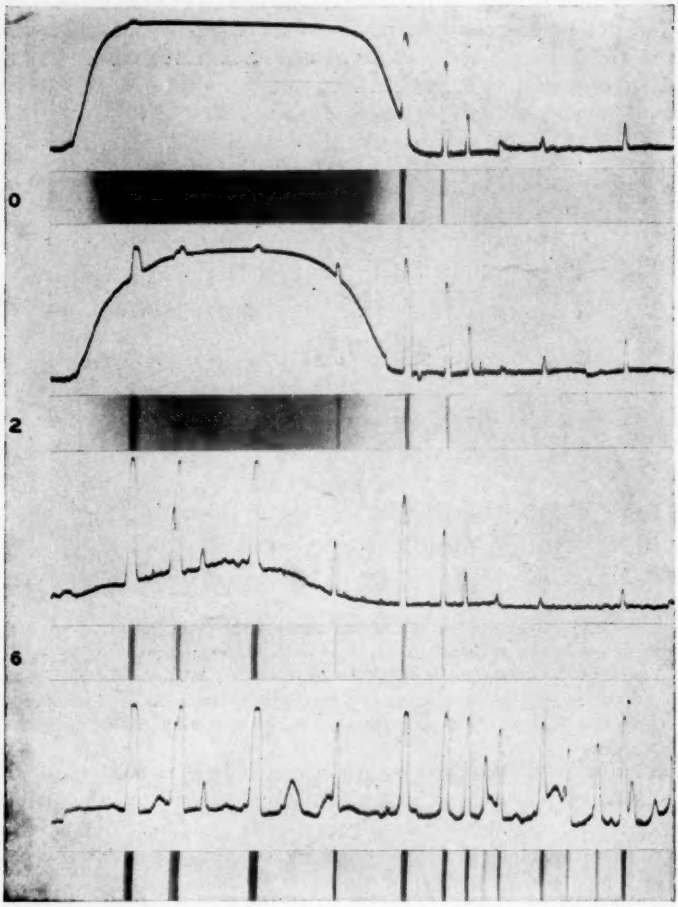

FIG. 3. Spectrograms with the corresponding microphotometer photographs of the fluorescent light emitted by indole-3-n-propionic acid during irradiation with ultraviolet light. The lower spectrum is that obtained when the solution of the acid is replaced with distilled water. The numbers at the left of each set of photographs give the number of hours the solution had been irradiated before the photographs were taken.

grams were taken with a Gaertner quartz spectrograph on Cramer contrast plates using a high frequency tungsten spark as the source of ultraviolet light. The solutions were contained in fused quartz precision cells during irradiation with the Victor mercury arc and while the spectra were being

photographed. The spectrograms, matched by the technique developed for this equipment by LOOFBOUROW (4), yield the curves shown in figure 4.

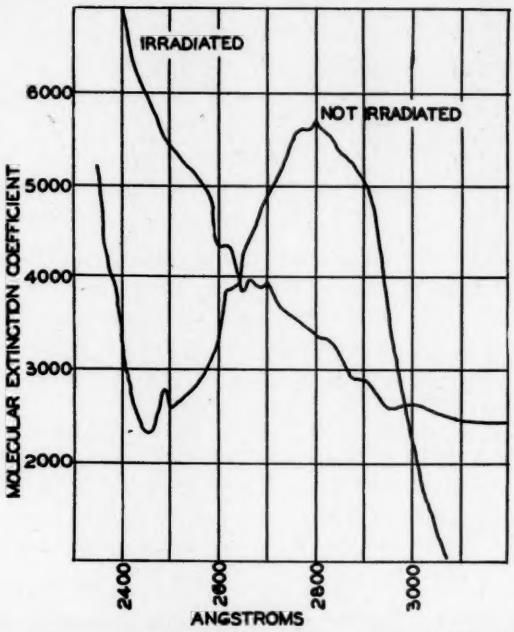

FIG. 4. Absorption curves of indole-3-n-propionic acid before and after 1 hour of irradiation.

EFFECT OF IRRADIATED INDOLE-3-N-PROPIONIC ACID ON PLANT ROOTS

It has been shown by HITCHCOCK (1) that the rooting of tomato and marigold cuttings can be retarded by proper concentrations of indole-3-n-propionic acid in water, and ZIMMERMAN and HITCHCOCK (7) have found that the aerial roots of tropical grapes were thickened abnormally and a large number of new roots developed when the root ends were immersed in growth solutions. Similar effects were observed by the writers when corn (Cross Golden Bantam = Purdue no. 51) and beans (Burpee's Stringless Green-pod bush bean) were grown in Knop's solution containing unirradiated acid. The effect was either absent or very much less in evidence when the acid used had been irradiated with ultraviolet light for several hours.

The seeds for this experiment were sprouted in clean, moist quartz sand. When the roots were from one-half to one inch long the seedlings were placed on cheesecloth stretched over a pan so that only the roots, projecting through the cloth, were immersed in water placed in the pan. After two

days of growth in this way they were transferred to pans containing the nutrient solutions.

A quantity of concentrated Knop's solution was prepared. This was divided into three equal portions. One portion was diluted with water so that the concentration of the salts in it were:

$Ca(NO_3)_2$	1.00 gm. per liter
KNO_3	0.25 gm. " "
KH_2PO_4	0.25 gm. " "
$MgSO_4$	0.25 gm. " "
$FePO_4$	trace

A second portion was diluted with an unirradiated solution of the acid so that the concentration of each of the salts was the same as that of the first portion but the solution contained 2 mg. of indole-3-n-propionic acid per liter in addition to these. A third portion was similarly diluted except that an irradiated solution of the acid was used. Each of these three solutions was then placed in a flat enameled pan holding four and one-half liters of solution. Each of the pans was provided with a wooden cover containing fourteen one-inch holes. Young bean seedlings were supported in these holes by cheesecloth spread across the holes in such a manner that the roots dipped into the solution. The roots were compared after the plants had been grown in a greenhouse for from five to seven days. The results are shown in figure 5.

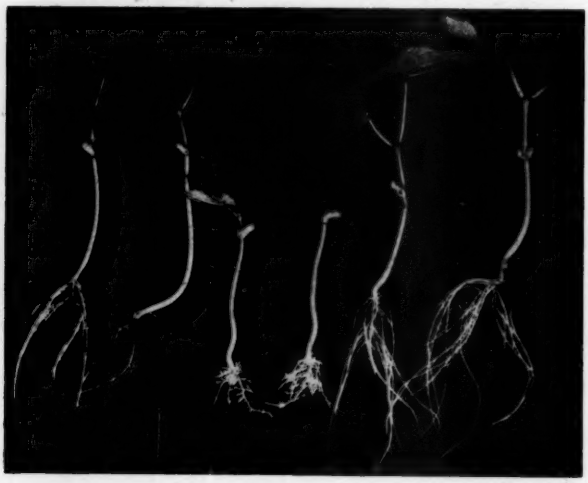

FIG. 5. Effect of indole-3-n-propionic acid upon the roots of bean plants. Left to right, irradiated acid, unirradiated acid, and control.

Roots of the plants placed in Knop's solution containing the non-irradiated acid solutions became thick and stubby and formed many branches. Frequently, the epidermal layer of the thickest roots split revealing scores of newly formed branching roots. The stems and leaves of the plants were usually stunted. On the other hand, roots of plants placed in Knop's solution containing the irradiated acid solutions grew in a normal or nearly normal manner. There was either no evidence or very little evidence of stunting in the stems or leaves of these plants.

Corn seedlings also produced abnormally thick and stubby roots in the unirradiated acid solution, and the leaves showed even greater stunting than that shown by the bean plants. The roots of seedlings grown in nutrient solutions to which a solution of irradiated indole-3-n-propionic acid had been added appeared normal (fig. 6).

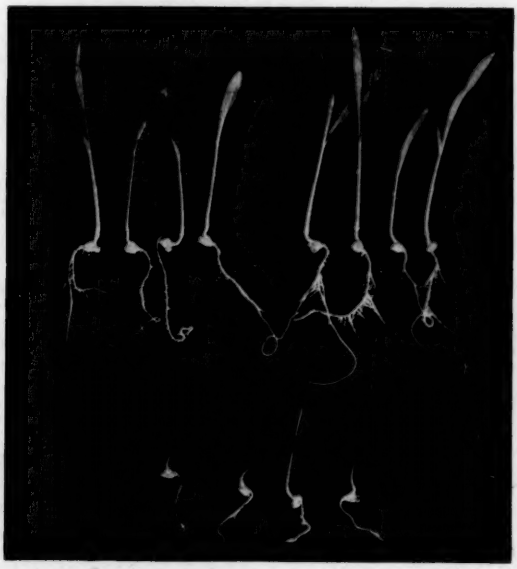

FIG. 6. Effect of indole-3-n-propionic acid upon the roots of corn plants. Upper left, irradiated acid; upper right, control; lower, unirradiated acid.

The amount of non-irradiated indole-3-n-propionic acid used in the solutions was much greater than that required for the formation of abnormally thick, stubby roots. This was demonstrated in an experiment in which the non-irradiated acid was added to the solution in several different concentrations; namely, 2, 1, 0.5, and 0.25 mg. per liter, respectively. There was little

or no difference in the appearance of the roots of bean plants grown in the solutions containing 2 mg. and 1 mg. of the acid per liter. The roots of the plants in solutions containing 0.5 mg. of the acid were thick but not as stubby as those receiving more acid, and the roots in the solution with only 0.25 mg. of the acid appeared normal in size but showed an abnormal amount of branching.

Bean seedlings grown in nutrient solutions to which had been added solutions of indole-3-n-propionic acid irradiated for periods of 2, 4, 6, and 8 hours showed that there seemed to be a progressive destruction of the acid during irradiation. Figure 7, which shows plants grown in solutions of

FIG. 7. Effect of indole-3-n-propionic acid solutions irradiated for various lengths of time upon roots of bean plants. Left to right, unirradiated, irradiated 2, 4, and 8 hours, control.

unirradiated acid, in acid solutions irradiated for 2, 4, and 8 hours, and in a nutrient solution, shows very clearly the gradual change which occurs in the acid in as far as it affects the development of normal roots. It was interesting to note that as long as there was any hint of the presence of a disagreeable odor the roots were not normal.

Discussion

The evidence here presented points to the fact that a chemical change takes place in indole-3-n-propionic acid during irradiation of the solution

with ultraviolet light. This is not surprising since tryptophan which is α-amino-indole-3-n-propionic acid has been shown to be destroyed by quartz lamp illumination (**2**). The destruction of both of these acids may be highly significant in plant life. We have shown that the destruction of indole-3-n-propionic acid is accompanied by a change in its action upon plant roots; therefore, a possible action of ultraviolet light upon the tryptophan within the plant may in some part explain the effect of ultraviolet light upon plants.

Since the authors were primarily interested in the physiological effect of indole-3-n-propionic acid, no particular effort was made to demonstrate just what change occurred in the material, although the changes in odor suggest that there may be first a breakdown to indole and then esterification of a product formed by a disruption of the indole ring. The odor of the product resulting after 8 hours of irradiation of the solution is strongly suggestive of that of methyl anthranilate which has a grape-like odor in dilute solutions and which has been shown to be present in grape juice (**5**). The idea that the indole ring in the acid is probably destroyed by ultraviolet radiation has further support (a) in that this breaking up by quartz-mercury lamp illumination of rings containing nitrogen in the nucleus has been demonstrated by LIEBEN and GETREUER (**3**) in the case of uric acid, xanthine, uracil, alloxan, hydantoin and α-hydroxynicotinic acid, and (b) in the appearance of the absorption spectra before and after irradiation of the solution. The absorption spectra of solutions of the unirradiated acid show absorption which is characteristic of indole derivatives (**6**). This absorption band is destroyed when the acid is irradiated, indicating that indole compounds have disappeared from the solution.

The difference in the appearance of the roots as the amount of acid in the solution was decreased was very marked. There was a tendency for the roots to grow longer with only the region near the tip becoming swollen. Short branching roots grew in this region. A comparison of the effect produced in the plants by solutions irradiated 8 hours and of the effect produced by various concentrations of the unirradiated acid shows that the 8-hour-irradiation period must produce a destruction of more than seven-eighths of the active groupings. The roots produced by plants grown in acid solutions irradiated 8 hours were like those produced by the control plants grown in Knop's solution except that, in some instances, they were not quite as long as those of the control plants. This slight inhibition of growth may be explained in that there was still a very small quantity of the original acid remaining in the solution, or in that the products formed during irradiation have a slight physiological effect upon the plants. Since there is not always a difference in root length of the control plants and plants in the irradiated acid solutions, the first suggestion seems to be the more logical.

This work with the unirradiated acid upon corn and bean plants extends the field of activity of indole-3-n-propionic acid to two new species and outlines a procedure which is easy to follow. The results are consistent whether the plants are grown individually in dark bottles or all in one pan.

Summary

Irradiation of indole-3-n-propionic acid with ultraviolet light from a mercury arc changes many of its physical properties and also changes its effect on the roots of plants.

The writers wish to express their thanks to Dr. D. A. Wells, Dr. J. H. Hoskins, and Dr. F. F. Heyroth for advice and assistance on this problem.

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FACTORS AFFECTING COLD RESISTANCE IN PLANTS

STUART DUNN

Introduction

In testing cabbage and other herbaceous plants for cold hardiness (**2**) a considerable amount of variation in the reaction of individual plants was found. A given group of ten or more similar plants would show freezing injury varying from total death through stages of intermediate injury to those unharmed, when subjected to average killing temperature.

It became of interest to determine if methods might be devised to produce plants of a more uniform nature and not showing so great a variation in hardiness within a group. Any factors, environmental or otherwise, that would aid in producing uniformity in that respect, would also help to increase the average group hardiness. In the present paper studies have been made on the effect of soil moisture, mineral nutrition, growth temperature, and selection.

TYSDAL (**7**) working with alfalfa and WORZELLA (**12**) with winter wheat found that the plants survived artificial freezing tests to a greater extent in dry soils than in wet soils. However, the plants were not grown continuously at these different soil moisture levels but the soil was merely allowed to become dry to a certain percentage or wetted to a high one shortly before freezing the plants. This was also true of the conditions under which alfalfa was tested by WEIMER (**8**), winter wheat by HILL and SALMON (**4**), and alfalfa by PELTIER and TYSDAL (**5**), where plants in the dry soil were injured more severely than those growing in wet soil. It seemed desirable to determine the effect of growing plants continuously from the seedling stage on at different soil moisture levels, not only for amount of survival but for uniformity of individual reaction.

The effect of mineral nutrition on hardiness, particularly with respect to the elements nitrogen, phosphorus, and potassium, has been extensively studied by WILHELM (**9, 10**). He investigated the effects on hardiness of growing various plants such as wheat, oats, barley, rye, tomatoes, and beans in sand cultures with high, normal, low, and minus amounts of these elements supplied in the nutrient solutions. In general high amounts of potassium gave greatest survival of freezing temperature, and low and normal nitrogen, and low and normal phosphorus the same. The plants receiving none of any one of the elements being studied were usually the poorest in survival. CRANE (**1**) has found that fertilization with nitrate of soda caused peach buds to be susceptible to winter injury.

Most of the work on effect of growth temperature on hardiness has been in the nature of hardening experiments for comparatively short periods of time when the plants were partly grown and in which rather extreme tem-

peratures were used either continuously or with alternations with higher temperatures. This was true of the work of TYDSDAL (7) and of TUMANOV (6). The former found that low temperatures alternating with higher ones produced hardier plants, and the latter found an intermediate continuous temperature more effective than a higher or lower one, but none of these as good as outside winter conditions. In the experiments reported here, the effect was determined of growing plants from seed continuously at different average mean temperatures. The effect of this sort of conditions has scarcely been reported, except for general observations on effects of different climatic conditions.

The value of vegetative selection as a means of increasing hardiness has, up to the present, not been given any consideration.

Experimentation

EFFECT OF SOIL MOISTURE

Cabbage plants were grown in soil in glazed pots, which for some of the experiments were of about one-half-gallon capacity, and for others one gallon. Air-dry soil was evenly tamped in them and the pots allowed to stand in water until the soil was saturated. On the basis of this weight of water as 100 per cent. saturation other percentages of 50, 60, 70, and 80 per cent. were figured, and, after drying down to these points, seeds were planted. Each pot was kept up to its assigned moisture content by frequent weighings and addition of water.

Several different series of plants were grown at each of the above constant moisture amounts. After reaching a good size, in 5 to 6 weeks, they were placed in a cold storage room at $-5°$ C. for 15 hours and observations made on survival.

The results of all these tests when summarized were found to be very contradictory and inconclusive and did not give any direct evidence that high or low soil moisture content influences hardiness. Thus the tabular data will be omitted. There were also encountered the usual variations in injury by plants within a group indicating that this method of growing plants does not produce similar behavior in hardiness among individuals treated alike.

EFFECT OF MINERAL NUTRITION

Cabbage plants were grown in sand in glazed 1-gallon pots, 3 plants per pot. Forty pots were used for the study of each nutrient and they were divided into two lots, one receiving a nutrient solution containing a low amount of the particular element being studied and the other a high amount of it. The composition of the different solutions used may be seen in table I. The solution contained a complete amount of the other necessary minerals in each case and the solutions were supplied to the plants every 48 hours,

TABLE I

TABLE OF DILUTIONS FOR NUTRIENT CULTURES

MILLILITERS OF ¼ MOL. SOLUTIONS OF SALTS MADE UP TO 1 LITER. DILUTED 10 TIMES AND APPLIED AT 200 ML. PER POT EVERY 48 HOURS

SALTS USED	NITROGEN		PHOSPHORUS		POTASSIUM	
	HIGH	LOW	HIGH	LOW	HIGH	LOW
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
$K H_2 PO_4$	80	80	75	25	75	25
$Ca (NO_3)_2$	120	20	75	75	75	75
$Mg SO_4$	80	80	75	75	75	75
$Fe Cl_3$	5	5	5	5	5	5
$Ca Cl_2$	.....	100	.....	.....	.....	.....
K Cl	.....	.....	.....	50	50	.....
$Na H_2 PO_4$	.....	.....	.....	.....	.....	50

with a thorough washing of the sand with water every week to remove accumulated deleterious substances. Thus the results on the effect of the lack or presence of each of these elements studied was obtained from a total of 60 plants for each treatment. The plants in all cases grew vigorously and were of uniform appearance. After they had attained a good size, usually at the age of about 2 months, they were tested for hardiness by exposure to a temperature of −5° C. for 24 hours. All plants were grown in a cool greenhouse at a temperature of 10° to 20° C.

The results appear in table II. The plants grown with high and low nitrogen showed striking differences in size and growth up to the time of chilling. Those with high nitrate were much larger and more vigorous, but the plants in each group were very uniform in appearance. Two separate series of tests were run on this element and in each case the percentage of those uninjured by the cold does not differ greatly in the two treatments. The percentage difference for one is exactly the reverse of the other. In all of these tests, as with previous work, individual variations were found.

Plants grown in the same manner as above with high and low potassium solutions did not show any visible differences in growth and size up to the time of chilling. The usual variations in individual reactions were found here also. The figures indicate a slight advantage in favor of the low potassium group, but the percentage difference would indicate no real significance.

Two different series of high and low phosphorus plants were grown and tested, the plants being of uniform, vigorous appearance. In the first series the usual individual differences were manifest, but in this case it was the high phosphorus group that showed the greater hardiness, more pronounced

TABLE II

EFFECT OF MINERAL NUTRITION ON HARDINESS OF CABBAGE

ELEMENTS IN NUTRIENT	PERCENTAGE OF SURVIVAL	
	HIGH	LOW
	%	%
Nitrogen	45	50
Nitrogen	60	54
Potassium	62	68
Phosphorus	45	35
Phosphorus	42	40
Fluorine	Fluorine added 35	No fluorine 42

than that apparent between the high and low series of the other two nutrients. The next year the other series was grown to confirm this difference if possible. Individual differences within the groups of similar treatment again appeared but the total difference between the high and low series was so slight as not to be significant. It is evident then that this element, like potassium and nitrogen, has but slight influence on hardiness.

The effect of fluorine on hardiness was tried because of the results reported by WILSON (**11**) on the effect of various halogen salts on some characteristics of tobacco plants. He found that fluorides in small amounts furnished to plants grown in soil increased the bound water content of the plant, which, according to some investigators, is associated with hardiness.

Accordingly cabbage plants were grown in sand culture in a similar way as for the other nutrient experiments, all of the plants being supplied with a complete nutrient solution. In addition half of the pots were treated with sodium fluoride. Beginning when the plants were 9 weeks old every other day each pot was supplied with 0.5 gm. of sodium fluoride dissolved in 200 cc. of its nutrient solution. About 8 days later the treated plants showed some slight signs of injury, such as yellow edges of leaves and reddened veins. On the twelfth day of treatment, the plants being good sized, both lots were chilled at $-5°$ C. for 20 hours. The results as given in the table show a greater total hardiness in the untreated plants, indicating that fluorine is of doubtful value in inducing hardiness.

EFFECT OF GROWTH TEMPERATURE

Although the percentage of soil moisture has but slight effect on hardiness of plants, growing them in a soil of constant moisture content is of great value in producing plants of uniform size and vigor. Therefore this

method was used in growing cabbage plants at a soil moisture content of 70 per cent. saturation in glazed 1-gallon pots and potato plants at one of 60 per cent. saturation in glazed 3-gallon pots. Sixty of the cabbage plants and 25 potato plants were grown in a warm greenhouse kept constantly between 15° and 30° C., and a like number in a cold greenhouse at 10° to 20° C. On the average there was 5° C. difference in temperature between the two houses at all times. The seed stock of the potatoes used in this experiment is of particular interest because of its previous treatment. Twenty-five tubers were progeny of plants that had been grown continuously in the warm house for three years and in this experiment were again grown in the warm house. The other twenty-five had a similar history and treatment in the cold house.

When the plants were of good size, at an age of 2 months in each case, they were chilled; the potato plants at −1.1° C. for 20 hours, and cabbage at −5° C. for 24 hours. The results on potato for 3 successive years and on cabbage for 2 years are summarized in table III. They indicate that a

TABLE III

EFFECT OF GROWTH TEMPERATURE ON HARDINESS

PLANTS	NO. OF CROPS	PERCENTAGE OF SURVIVAL	
		PLANTS GROWN AT 10°–20° C.	PLANTS GROWN AT 15°–30° C.
		%	%
Potato	1	94	32
	2	59	38
	3	62	45
Cabbage	1	35	29
	2	70	56

colder growth temperature is effective in producing a group of plants more hardy than those grown at a warmer temperature. Nevertheless, here as in the case of plants grown under other variations of environmental conditions there was no uniformity of behavior of individuals in any one group of plants.

EFFECT OF SELECTION BY VEGETATIVE PROPAGATION

As has been emphasized hitherto, in any given lot of plants there is a marked tendency toward individual variation in hardiness from total killing to no injury. It has been further shown that constancy of environmental factors, such as soil moisture content and growth temperature, does not produce greater uniformity in that respect.

It seemed desirable then to determine if these individual variations could be isolated or passed on from one generation to the next; that is, whether or not a uniformly hardy strain of plants could be produced by selection. Because of genetical difficulties and other complications involved in growing plants from seed, vegetative propagation was used. For this work *Bryophyllum* and Jerusalem artichoke were used as they are easily propagated vegetatively, the former by leaf cuttings and the latter by tubers.

A large number of *Bryophyllum* plants were started by leaf cuttings in sand from ordinary greenhouse stock. The plants were grown in soil in pots until of fair size and then chilled at −1.1° C. for 15 hours. This was found to be about right for the threshold killing temperature, as it was for potato plants. When first subjected to this temperature a large number of the plants were killed, but cuttings were made from the survivals and other plants of the second generation raised from them. When these had all attained sufficient size they were again chilled and the process repeated for several generations. The time required for each generation to be grown to a certain size varied from 4 to 6 months in most cases and thus they were spread out over all seasons of the year, but at a warm temperature in all cases. Along with them were chilled a considerable number of plants of ordinary stock started from cuttings simultaneously with those of the selected stock and grown to the same age, under the same conditions, for comparative purposes. The results of these tests for six generations are summarized in table IV.

TABLE IV

EFFECT OF SELECTION BY VEGETATIVE PROPAGATION ON HARDINESS OF *BRYOPHYLLUM*

No. of generations	Stock selected from hardy individuals		Unselected stock	
	No. of plants chilled	Survival	No. of plants chilled	Survival
		%		%
1	.....	.....	40	15
2	63	73	100	32
3	102	77	121	50
4	135	65	100	53
5	221	54	129	36
6	271	40	250	39

These results indicate that it is possible to build up temporarily a resistant strain by vegetative propagation, with the high point in this case coming in the third generation, but eventually the plants will revert back

toward the original condition. This is evident from the close agreement of the percentage of plants surviving in the two groups of plants in the sixth generation. Although the percentage of survival of the unselected stock in the third and fourth generations is rather high, the average of all the figures for this stock is 37, which again is very close to the final value of 40 per cent. survival of the selected plants.

The results of tests with Jerusalem artichoke were very similar to those on *Bryophyllum,* except that the reversion to the original status comes in fewer generations.

An explanation of the reason for this temporary rise in hardiness in a group of plants treated in this manner is not readily forthcoming. Comparison of these results to the time of year during which the generations were grown reveals nothing consistent as regards seasonal variations to which it may be due. Probably it is due to a stimulus or shock from the treatment which wears off after a time. Such reactions are not unknown among plants. HARVEY (3) suggests that hardening in cabbage is a cold shock response, in that subjecting plants to cold for a few hours daily is just as effective in hardening them as continuous cold and that a few days of this treatment is as effective, or more so, than many days. A sort of fatigue is also evidenced by the sensitive plant, *Mimosa,* after repeated contact or jarring stimuli, if the repetitions are close together.

It seems then, that selection of surviving plants and their vegetative propagation is not enough to hold hardiness at a high level. Probably in combination with some other factor of the environment, such as temperature of growth, the method might be more successful in producing a hardy strain.

Summary

1. An outstanding characteristic of herbaceous plants, tested for hardiness by freezing, is the individual variation in reaction in any given group of plants. Experiments were tried to eliminate these variations and to increase the average resistance of a group of plants by varying some of the environmental conditions, such as soil moisture, mineral nutrients, growth temperature, and by means of vegetative propagation of survivals of lengthy exposure to cold.

2. Growing cabbage plants at different constant soil moisture levels did not eliminate variations of hardiness within a group, and did not increase the average hardiness of any group.

3. High and low amounts of the nutrients nitrogen, phosphorus, and potassium did not affect degree or uniformity of hardiness of cabbage.

4. A cool growth temperature for potato and cabbage did not eliminate individual variations within a group, but did cause a greater average survival from freezing than with those grown at a warm temperature.

5. Vegetative propagation of survivals of cold exposure in groups of *Bryophyllum* and Jerusalem artichoke plants for several generations indi-

cated that the average hardiness within such a group could be increased temporarily, but eventually fell back to the original condition. Probably the effect of some other factor acting in cooperation with selection, such as a cool growing temperature, is required for holding the average survival at a high level.

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GAS CONTENT OF CRANBERRIES AND POSSIBLE RELATIONSHIP OF RESPIRATORY ACTIVITY TO KEEPING QUALITY[1]

WM. B. ESSELEN, JR. AND C. R. FELLERS

(WITH ONE FIGURE)

Introduction

This investigation was carried on to determine the changes in the composition of the internal atmosphere and in the catalase activity of cranberries as affected by variety and temperature while the fruit is in storage.

Only a few papers have been published on the composition of the internal atmosphere of fruits. In much of the work that was carried on no attempt was made to correlate the results obtained with the keeping quality of the fruit. In this investigation the relationship between the experimentally determined keeping quality of cranberries and the composition of the internal atmosphere is noted. No previous published report on the composition of the internal atmosphere of the cranberry was found.

Equipment for collecting and analyzing gas

Since the methods used by previous workers were not suitable for this particular investigation it was necessary to devise a method for collecting the gas contained in the voids of cranberries. The following method for collecting gas from small fruit is rapid and sufficiently accurate for this work.

The gas-collecting apparatus consists of a glass funnel inverted in a 1-liter beaker, containing approximately 500 cc. of water, freshly boiled, to expel any dissolved gases. The sample of cranberries, approximately 70 gm., is placed in the beaker of water and the inverted funnel is placed over the fruit. A length of rubber tubing connects the stem of the funnel with the gas-analysis apparatus.

For the actual analysis of the gas a modification of the original Orsat apparatus was used. This apparatus consisted of a gas-measuring burette and two bubbling absorption pipettes. The first pipette, used to absorb carbon dioxide, contains a 12 M solution of sodium hydroxide, while the second pipette used to absorb oxygen, contains an alkaline pyrogallic acid solution.

The gas-collecting and analyzing apparatus is set up as shown in figure 1. By means of the water-level bottle the air in the funnel is removed, and the funnel and tube connecting the funnel with the Orsat apparatus are filled with "gas-free water" from the beaker. The water in the beaker is heated

[1] Contribution no. 253, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.

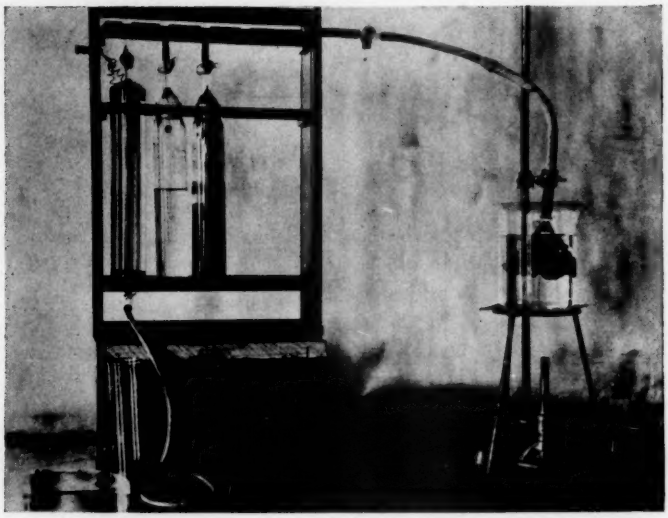

FIG. 1. Apparatus for collecting and analyzing gas.

until all of the gas is expelled from the voids and intercellular spaces of the cranberries. This gas collects in the upper part of the funnel. As soon as the gas is collected it is drawn into the gas-measuring burette by means of the water-level bottle. The gas is allowed to stand until it has come to the temperature of the apparatus, and then its volume is determined by holding the water-level bottle so that the water level is the same in the bottle as in the burette. The sample of gas is passed into the carbon dioxide absorption pipette, and then passed back into the measuring burette. The absorptions are continued until a constant reading is obtained in the measuring burette. The volume is read and the difference from the original volume represents the volume of carbon dioxide contained in the sample. After the volume of carbon dioxide is determined, the volume of oxygen is determined in a similar manner by bubbling the gas into the oxygen absorption pipette. The residual gas after the absorption of carbon dioxide and oxygen is calculated as nitrogen. The volume of each of the gases is expressed in terms of percentage of total volume of gas.

Experimentation

EFFECT OF FREEZING AND SUBMERGENCE ON THE COMPOSITION OF INTERNAL ATMOSPHERE OF CRANBERRIES

In order to determine the effect of various environmental factors on the composition of the internal atmosphere of cranberries, several samples of

TABLE I

EFFECT OF FREEZING AND SUBMERGENCE ON COMPOSITION OF INTERNAL ATMOSPHERE OF CRANBERRIES. SAMPLES FROM MILLIS, MASSACHUSETTS

SAMPLE	DATE HARVESTED	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO_2/O_2)	HISTORY OF SAMPLE
			%	%	%		
1	Sept. 4–10	3	16.06	7.52	76.41	0.468	Stored in ventilated barn for 25 days
2	Oct. 30	3	9.97	10.75	79.28	1.075	Dropped on bog when sample 1 was raked. When fruit was gathered it was floating in water, under ¼ inch of ice. Fruit in good condition
3	Oct. 25	7	13.24	10.57	76.19	1.252	Left on vines until time of picking. Under water 10 days. Fruit in good condition
4	Oct. 25	3	11.90	5.18	82.92	0.435	Left on vines until time of picking. Were all above the water on the bog and had been frosted. Fruit quite soft
5	Oct. 30 Sound fruit	4	11.54	11.12	77.33	0.972	Left on vines until time of picking. Bog frozen over and fruit above the ice. About ⅓ of sample was frosted and the other ⅔ in apparently good condition
5	Oct. 30 Frosted fruit	2	12.48	5.02	82.47	0.404	

fruit were collected from a natural cranberry bog, at Millis, Massachusetts. The cranberries were of the Howes variety. Summaries of the composition of the internal gas and the carbon dioxide-oxygen ratio, as well as the environmental conditions to which each sample was subjected, are presented in table I.

Considering the carbon-dioxide ratio as indicative of the rate of respiration, the cranberries which were left on the vines had a much higher rate of respiration than those which had been harvested about a month earlier and placed in storage. Since a high rate of respiration is detrimental to fruit, it would seem advisable not to leave the cranberries on the vines too long after they are mature.

TABLE II

COMPOSITION OF INTERNAL ATMOSPHERE OF EARLY BLACKS STORED AT 3° C. (37.4° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO_2/O_2)	CONDITION OF FRUIT
		%	%	%		
0	10	12.73	6.12	81.04	0.48	Good
7	4	19.49	2.75	77.85	0.14	"
14	4	16.75	3.20	80.04	0.19	"
26	7	11.55	2.89	85.54	0.25	"
32	7	16.26	4.05	79.68	0.24	"
46	2	18.33	2.26	79.40	0.12	"
52	2	17.78	1.71	80.50	0.09	Fruit becoming soft and decayed
74	1	15.81	1.86	82.33	0.12	Fruit approximately 50% decayed
84	1	15.37	5.59	79.04	0.36	Fruit approximately 50% decayed

Of the cranberries which were raked early in September, the fruit which was left on the bog and submerged in water had a much higher rate of respiration a month after picking than those cranberries which had been in storage during this time.

Of the cranberries which were left on the vines, those which were submerged in water showed a higher rate of respiration than those which were above water and in the air. It is possible to correlate these results with those obtained by FRANKLIN (1), and also by WAKABAYASHI (9), who found that submergence weakened cranberries and made them more susceptible to disease and breakdown. A correlation of results indicates that the weakened condition of cranberries which have been submerged in water is due to the

increased rate of respiration. In cranberries which have been frosted the rate of respiration is materially reduced.

Composition of internal atmosphere as affected by variety, temperature, and period of storage

In order to determine the variations in the composition of the internal atmosphere of cranberries throughout a definite storage period, and at different temperatures, two varieties of cranberries, Early Blacks and Howes,

TABLE III

Composition of internal atmosphere of Early Blacks stored at 24° C. (75.2° F.)

Days in storage	No. of determinations	Oxygen	Carbon dioxide	Nitrogen	Carbon dioxide-oxygen ratio (CO_2/O_2)	Condition of fruit
		%	%	%		
0	10	12.73	6.12	81.04	0.48	Good
7	7	12.53	7.89	79.13	0.62	"
14	7	9.21	12.16	78.62	1.32	Fruit becoming soft, rot setting in
21	7	6.66	12.89	80.44	1.93	Fruit soft and shriveled
28	7	5.10	12.02	82.86	2.35	High percentage of fruit decayed and soft
35	7	10.13	10.47	79.39	1.03	Fruit very soft
40	3	11.64	7.79	80.56	0.67	Fruit very soft and shriveled
46	3	9.49	11.28	79.23	1.13	Fruit very soft and shriveled
53	3	9.27	9.32	81.40	1.00	Fruit very soft
61	2	10.33	7.80	81.87	0.75	
68	1	7.33	10.00	82.67	1.36	Fruit shriveled and dried
78	1	8.59	8.59	82.82	1.00	Fruit 100% soft, shriveled, rotten, and dried

were stored in a cold storage room at 3° C. and in a warm room at 24° C. Samples of the fruit were taken at weekly intervals and the internal gas was analyzed. In order to have representative samples, cranberries were taken from various parts of the storage container and mixed together to make a uniform sample. All of the cranberries used in this experimental work came from Wareham, Massachusetts.

The results of the weekly analyses of the samples of cranberries are given in tables II, III, IV, and V. Results are calculated as percentage by

volume of oxygen, carbon dioxide, and nitrogen, and the carbon dioxide-oxygen ratio is given.

Discussion of results

The temperature of storage has a marked effect on the keeping quality of cranberries. The Early Blacks stored at 24° C. broke down and decayed in approximately twenty days, while those stored at 3° C. remained in good

TABLE IV

COMPOSITION OF INTERNAL ATMOSPHERE OF HOWES STORED AT 3° C. (37.4° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO_2/O_2)	CONDITION OF FRUIT
		%	%	%		
0	11	14.59	8.32	77.08	0.57	Good
7	4	13.94	8.80	77.25	0.63	"
14	1	15.66	7.83	76.51	0.49	"
24	3	12.42	7.98	79.71	0.64	"
27	4	15.92	6.53	77.64	0.41	"
37	6	13.65	9.32	77.02	0.68	"
44	3	13.49	8.80	77.70	0.65	"
50	3	15.57	7.24	77.18	0.46	"
58	3	13.47	5.28	81.22	0.39	"
76	2	10.40	6.82	82.77	0.65	"
81	3	15.95	5.75	78.29	0.36	Fruit slightly soft
87	3	15.30	6.08	78.61	0.39	" " "
109	2	14.94	6.63	78.42	0.44	Fruit slightly soft, otherwise in good condition
116	1	13.43	5.97	80.60	0.44	Fruit slightly soft, otherwise in good condition
151	2	14.19	5.92	79.88	0.41	Fruit slightly soft, otherwise in good condition
159	5	15.55	5.86	78.57	0.37	Fruit slightly soft, otherwise in good condition
168	3	15.45	5.27	79.25	0.34	Fruit in fairly good condition
197	3	15.49	4.79	79.71	0.31	Fruit 50 to 70% soft and unmarketable

condition for over three months. At a temperature of 24° C. the Howes also broke down in approximately twenty days; and at this warm temperature their keeping quality was no better than that of the Early Blacks. However, at a temperature of 3° C. the Howes remained in good condition for seven months.

Throughout all of the tests the internal atmosphere of the Howes had a much higher carbon dioxide content and a correspondingly lower oxygen

content than did the Early Blacks. Hence, the carbon dioxide-oxygen ratio of the Howes was considerably greater than that of the Early Blacks. These results indicate that the keeping quality of cranberries may vary with the carbon dioxide content and the carbon dioxide-oxygen ratio.

The nitrogen plus inert gas content of the internal gas of the cranberries is similar to that of the atmosphere, about 79 per cent., except when the fruit is respiring at a very rapid rate. When the respiration rate is high

TABLE V

COMPOSITION OF INTERNAL ATMOSPHERE OF HOWES STORED AT 24° C. (75.2° F.)

DAYS IN STORAGE	NO. OF DETERMINATIONS	OXYGEN	CARBON DIOXIDE	NITROGEN	CARBON DIOXIDE-OXYGEN RATIO (CO_2/O_2)	CONDITION OF FRUIT
		%	%	%		
0	11	14.59	8.32	77.08	0.57	Good
6	9	7.21	13.66	79.21	1.89	Slightly soft
13	10	6.24	16.95	76.80	2.71	Fruit becoming soft
21	10	5.39	17.62	76.98	3.27	Fruit quite soft, rot setting in, unmarketable condition
28	7	5.14	18.49	76.21	3.59	Softness and rot increasing
37	7	4.64	17.24	78.11	3.71	Large percentage of fruit very soft
44	7	5.28	17.90	76.81	3.38	Most of fruit very soft
50	6	5.61	17.07	77.31	3.04	Most of fruit very soft
58	6	5.53	16.17	78.27	2.92	Fruit very soft
66	3	7.53	12.93	79.53	1.71	Fruit very soft
73	2	6.14	11.25	82.85	1.83	Fruit badly shriveled
80	1	5.73	11.46	82.81	2.00	Fruit in decayed condition

the increased carbon dioxide content apparently replaces some of the nitrogen. But in general it may be said that the precentage of nitrogen in the internal atmosphere of cranberries is quite stable. When the cranberries have stopped respiring, or the cells are dead, the composition of the internal atmosphere approximates that of the outside atmosphere, *i.e.*, 79 per cent. nitrogen and 21 per cent. oxygen.

When stored at a warm temperature, the Howes showed a very rapid increase in the carbon dioxide content and a correspondingly rapid decrease after the peak was reached. Under similar conditions the carbon dioxide

content of the Early Blacks showed a more gradual increase and decrease and the peak was lower than in the Howes variety. MAGNESS (5), KIDD and WEST (4), and HARLEY and FISHER (2) obtained similar results from their studies on the internal atmosphere of apples.

In the cranberry there is a definite amount of respirable material which must be used up during respiration, before internal breakdown sets in. If the fruit is stored at a high temperature this material is used up more rapidly, as is shown by the carbon dioxide-oxygen ratios. In a similar manner it is shown that when cranberries are stored at low temperatures this respirable material is used up very gradually with the result that the life of the cranberry is greatly prolonged.

It is interesting to note that the Early Blacks stored at 24° C. showed a secondary peak in the carbon dioxide content and in the carbon dioxide-oxygen ratio. This secondary peak occurred after the fruit was very soft and decomposed. The odor of the cranberries made it quite evident that fermentation was taking place within the fruit. In all probability this increase in the carbon dioxide content of the internal gas was due to the action of microorganisms on the decomposed fruit.

The data obtained from these tests indicate that by a study of the carbon dioxide-oxygen ratios, based on the composition of the internal atmosphere of cranberries, it is possible to determine the length of time that cranberries may be kept in cold storage before internal breakdown sets in, and the fruit is in an unmarketable condition. This information may be obtained by running a test on cranberries stored at a high temperature for approximately ten days in the fall, when the fruit is first placed in storage. By comparing the carbon dioxide-oxygen ratios it is possible to forescast ahead approximately how long cranberries may be kept in storage. Such a forecast of the keeping quality of cranberries should be of value to canneries and people using cold storage. The writers propose also to check this method against the "spore load" and incubation tests for keeping quality as described by STEVENS (8). The present studies supplement the reports on respiration in cranberries during storage by MORSE (6) and on the spoilage of cranberries after harvest by SHEAR, STEVENS, WILCOX, and RUDOLPH (7). In general, these results are in agreement with the findings of these investigators.

CATALASE ACTIVITY OF CRANBERRIES STORED AT 3° C. AND 24° C.

In conjunction with the study of the changes in the composition of the internal atmosphere of cranberries during storage the catalase activity of the cranberries was determined at weekly intervals. Two methods were used in the determination of catalase activity. The first method used was the potassium permanganate titration method, by which the hydrogen per-

oxide not decomposed by the catalase is determined by titrating with potassium permanganate. The amount of potassium permanganate solution used varied inversely with the catalase activity. This method was discontinued because the tannins and pigments present in the fruit also reduce the permanganate solution, and because the results obtained are not a true indication of the actual catalase activity.

The second method for catalase determination was very satisfactory. The principle of the method is the volumetric liberation of oxygen from hydrogen peroxide as described by HAWK and BERGEIM (**3**). While cranberries show a moderate catalase activity, there was no correlation between catalase and respiratory activity at 3° C. but there was a direct correlation at 24° C. In general, a high catalase activity is associated with poor keeping quality. There is a sharp increase in activity a short time before cranberries begin to show structural breakdown in cold storage. The volume of oxygen liberated from hydrogen in Howes or Early Black varieties stored at 3° C. varies from 2 to 4 cc., while at 24° C. this value is somewhat higher. The data were not sufficiently impressive to warrant further experimental work though four different lots of cranberries were examined at weekly intervals for several months.

Summary

1. Submergence causes an increased rate of respiration in cranberries and an increase in the carbon dioxide content of the internal atmosphere. It is probable that the increased rate of respiration weakens the cranberry and makes it more susceptible to fungus disease, as has been observed by FRANKLIN (**1**) and also by WAKABAYASHI (**9**).

2. Frosting reduces the rate of respiration in cranberries.

3. There is an increase in the respiration of cranberries if they are left on the vines after the usual picking time or maturity.

4. The temperature of storage has a marked affect on the keeping quality of cranberries. Cranberries stored at 24° C. (75.2° F.) became soft and partially decayed within twenty days.

5. The carbon dioxide content and the carbon dioxide-oxygen ratio vary directly with the keeping quality of the cranberries.

6. The nitrogen content of the internal atmosphere of cranberries is relatively constant and approximates that of the atmosphere. The carbon dioxide and oxygen contents of the internal gas vary with the rate of respiration.

7. Cranberries contain a definite amount of respirable material which must be used up before cranberries show physical breakdown in storage. When there is a high rate of respiration these expendable energy reserves are used up more rapidly and the life of the cranberry in storage is correspondingly decreased.

8. By means of the carbon dioxide-oxygen ratio it is possible to forecast with fairly good accuracy several months in advance the keeping qualities of cranberries in cold storage.

9. There is no significant correlation between catalase activity and respiratory activity of cranberries in storage.

10. The potassium permanganate titration method for the determination of catalase activity in cranberries is inaccurate because of interfering substances which are present in the cranberry.

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NATURE OF THE BLACKMAN REACTION IN PHOTOSYNTHESIS

ROBERT EMERSON AND LOWELL GREEN

(WITH THREE FIGURES)

Introduction

Among those writing on the mechanism of photosynthesis, which include WARBURG (**7**), WILLSTÄTTER (**9**), and FRANCK (**3**), the opinion is widely held that the Blackman reaction, which limits the rate of assimilation at high light intensities and carbon dioxide concentrations, involves the decomposition of a peroxide, formed in a preceding photochemical reaction, by the enzyme catalase. The best experimental support of this theory is found in the work of WARBURG and UYESUGI (**8**), and YABUSOE (**11**), who compared the temperature coefficients and sensitivity to several inhibitors for the Blackman reaction and for the decomposition of added hydrogen peroxide. In these respects they found certain similarities between the two processes. Such differences as they found were readily explainable, they believed, on the ground that while the same enzyme, catalase, played a part in both cases, the substrate was probably different, being very likely some organic peroxide, in the case of the Blackman reaction, rather than hydrogen peroxide.

Largely as a result of this work, WARBURG (**6**) gave up his "acceptor theory" in which the Blackman reaction preceded the photochemical reaction, and in agreement with WILLSTÄTTER and STOLL (**10**) regarded the Blackman reaction as involving the decomposition of a peroxide previously formed in the photochemical reaction. The writers have made further comparisons between peroxide decomposition and the Blackman reaction, and believe WARBURG's conclusion should be reconsidered, especially in view of the prominent part played by peroxide in the recently proposed chemical mechanisms for photosypthesis. GAFFRON and WOHL (**5**), in a theoretical paper, have objected that the energy available for the assimilatory process is insufficient to permit the formation of hydrogen peroxide as an intermediate product; but this objection seems to us of minor importance in the present discussion, because no definite claim has been made that the peroxide formed must be *hydrogen* peroxide. WILLSTÄTTER and STOLL (**9**) are more inclined to the opinion that the action of catalase in photosynthesis is on some peroxidic compound other than hydrogen peroxide. GAFFRON and WOHL also believe the formation of some sort of peroxide is hardly to be avoided, and suggest, on purely theoretical grounds, a peroxide of organic nitrogen, still regarding catalase as responsible for the freeing of the oxygen produced in photosynthesis.

In support of YABUSOE's observation that the Blackman reaction and peroxide decomposition are both linear functions of temperature, GAFFRON and WOHL cite (incorrectly) FRENCH (4) as confirming this for the Blackman reaction. But FRENCH reported no experiments on assimilation. His measurements show that peroxide decomposition varies in accordance with the Arrhenius equation, and not linearly with temperature.

Experimentation

The writers have compared the rates of the Blackman reaction and decomposition of hydrogen peroxide by the same organism used by WARBURG (*Chlorella pyrenoidosa*) and by a second species of lower assimilatory activity, *C. vulgaris*. Some confusion may arise as to the names of these organisms, because WARBURG has sometimes referred to his as *C. vulgaris*. The taxonomic differences between the two species are unsatisfactory and difficult to establish, but there can be no doubt as to their physiological differences, and the one used by WARBURG certainly corresponds with the one we call *C. pyrenoidosa*. In cultures of average density (1 mm.^{3} cells per ml. of fluid) and at 25° C. it shows a maximum assimilation of about 25 times its own volume of carbon dioxide per hour. *C. vulgaris* grows much more slowly, and shows under similar conditions a rate of assimilation only ten to fifteen times its own volume per hour. We have cultivated both species in pure culture for over ten years, and have never failed to find these physiological differences, as well as many others of which no mention has been made in published papers because they have as yet no clear bearing on problems of photosynthesis. The two species were last determined by Fräulein CANNABAEUS, in the laboratory of Professor KOLKWITZ, Berliner Institut für Wasser-Hygiene, in 1927.

The Blackman reaction is the name given to the rate-determining process when photosynthesis is saturated with both light and carbon dioxide. We define its rate as the number of cubic millimeters of oxygen produced per hour by one cubic millimeter of cells under saturating conditions. This was measured manometrically in the usual way, using rectangular vessels of the type shown in figure 1. Such vessels were filled with a suspension of cells

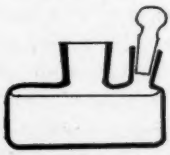

FIG. 1. Rectangular vessel used for determining rate of Blackman reaction.

sufficiently dense to give satisfactory readings on the manometer. The volume of cells used ran from about 2 mm.^{3} for the more active suspensions

to 10 or 15 mm.^{3} for the less active ones. As suspending fluid, either Knop's solution saturated with 5 per cent. carbon dioxide in air, or a carbonate mixture, was used. This mixture was prepared fresh for each experiment by mixing 85 parts M/10 potassium bicarbonate with 15 parts M/10 potassium carbonate. In either of these suspending fluids, large changes in carbon dioxide concentration resulted in only minor changes in the rate of assimilation. Saturating illumination, in which large changes in intensity produced no significant changes in rate of assimilation, was provided by a row of closely spaced 60-watt incandescent lamps whose tops were about 10 cm. from the bottoms of the vessels. A series of readings in the light was customarily followed by a dark reading, and the rate of respiration thus obtained was applied as a correction to the rate of photosynthesis. As a rule, the respiration correction is of no importance in establishing the rate of the Blackman reaction, because photosynthesis at light saturation is about twenty times as great as respiration. Nothing would be changed in our conclusions if the respiration correction were omitted altogether.

The rate of decomposition of hydrogen peroxide was measured in darkness, in vessels of the type shown in figure 2. Three ml. of cell suspension

FIG. 2. Conical vessel used for determining rate of decomposition of hydrogen peroxide.

were filled into the main space, 0.3 ml. of 5 per cent. potassium hydroxide into the central well, and 0.3 ml. of M/30 hydrogen peroxide into the side bulb. Knop's solution was used as suspending fluid for the cells, and the peroxide solution was prepared by diluting Merck's "superoxol."

After attainment of temperature equilibrium, the peroxide was mixed with the cell suspension, and the oxygen production followed manometrically at 5-minute intervals, usually for about half an hour. The amount evolved in the first 15 minutes was used as a basis for computing the rate, which we have expressed in the same units as the Blackman reaction, to make the two rates readily comparable: mm.^{3} of oxygen produced per hour per mm.^{3} of cells. The experimental set-up and method of handling the data are essentially the same as were used by WARBURG and his collaborators, and, as we shall see, give much the same results. It is important to use a

minimum amount of cells in proportion to the amount of peroxide, to avoid large changes in peroxide concentration during the first 15 minutes. WARBURG's practice was to use 50 mm.^{3} cells in 10 ml. fluid for each determination. The writers have found it practicable to use somewhat fewer cells, from 2 to 10 mm.^{3} in 3 ml. of fluid, depending on their activity. In our experiments there is no indication of a decline in rate of peroxide decomposition during the first 15 minutes, except in the case of *C. vulgaris* at 20° C. and over. This was due to gradual injury of the cells by the peroxide rather than to any fall in peroxide concentration, as we were able to show by following the process for longer periods of time (3 hours) and at lower concentrations of peroxide. For *C. vulgaris* it would be more favorable to use M/600 instead of M/300 peroxide, especially at 20° C. and above. This concentration is less suitable for *C. pyrenoidosa,* and the writers thought it better to use a standard technique throughout, especially since the injury to *C. vulgaris* by peroxide at the lower temperatures is so slow that it is negligible in our experiments. In our tables, the rates for *C. vulgaris* above 20° C. are probably 10 to 20 per cent. lower than they should be, but this alters nothing in our conclusions, and tends only to minimize, rather than magnify, the differences on which they are based, because *C. vulgaris* always has a much higher rate of peroxide decomposition and a smaller Blackman reaction than *C. pyrenoidosa.*

We have followed WARBURG's practice of making no correction for respiration in the peroxide experiments, on the supposition that peroxide, rather than molecular oxygen, would be used by the cells as a source of oxygen for respiration under these conditions (WARBURG and UYESUGI, **8**).

Results

For cultures of equivalent density, *C. vulgaris* regularly shows a rate of the Blackman reaction about half as great as *C. pyrenoidosa,* but a rate of peroxide decomposition up to ten times as great. Yeast cells, though they cannot be said to have any Blackman reaction, show a rate of peroxide de-

TABLE I

RATES OF BLACKMAN REACTION AND PEROXIDE DECOMPOSITION AT 22° C. FOR *Chlorella vulgaris, C. pyrenoidosa,* AND YEAST (*Saccharomyces cereviseae*). RATES EXPRESSED IN MM.^{3} OXYGEN EVOLVED PER HOUR PER MM.^{3} OF CELLS

	C. VULGARIS	*C. PYRENOIDOSA*	YEAST
Rate of Blackman reaction	16.4	26.7	.....
Rate of peroxide decomposition	38.3	4.1	68.8

composition about twice as great as *C. vulgaris*. Some figures are shown in table I. Exact comparison of the *C. pyrenoidosa* rates with WARBURG's is not possible, because UYESUGI and YABUSOE do not specify the exact amount of cells used for individual experiments, but an approximate comparison can be made from their general statements. For peroxide decomposition, they report from 20 to 40 mm.^{3} oxygen evolved by 50 mm.^{3} of cells in the first 15 minutes. In the units of table I, this means a rate of 6.4 to 12.8. For the Blackman reaction they report rates of 8 to 25, in the units of table I. The value of 8, taken from one of UYESUGI's tables, appears rather low to the writers, due perhaps to his use of very dense suspensions—too dense, in our opinion, for adequate light saturation. However, Mr. SARGENT's experiments, conducted in this laboratory, show that rates as low as 8 may be obtained with cells from old and very dense cultures of *C. pyrenoidosa*. It is to be noted that WARBURG's experiments were at 20° C. while the rates in table I are for 22° C., a difference of minor importance.

In table II are rates for the Blackman reaction and peroxide decomposition for *C. vulgaris* cells grown in glucose cultures with different amounts

TABLE II

COMPARISON OF RATES OF BLACKMAN REACTION AND PEROXIDE DECOMPOSITION FOR *C. vulgaris* CELLS CULTURED IN GLUCOSE WITH DIFFERENT AMOUNTS OF IRON. FIGURES ARE FOR 20° C., AND SHOW MM.^{3} OXYGEN PRODUCED PER HOUR PER MM.^{3} OF CELLS

	IRON ADDED TO CULTURE	NO IRON ADDED	IRON REMOVED BY AUTOCLAVING WITH $CaCO_3$
Rate of Blackman reaction	5.7	3.5	< 1
Rate of peroxide decomposition	16.0	18.0	16.4

of iron, by the method of EMERSON (1). The diminished rate of the Blackman reaction in the iron-poor cells is not accompanied by a lower rate of peroxide decomposition.

It will be noted that these rates of peroxide decomposition are lower than for *C. vulgaris* in table I. This is due in part to the use of glucose in culturing the cells for the experiments in table II. Glucose in concentrations of about 1 per cent. inhibits the peroxide decomposition 15 to 35 per cent., though it has no direct effect on the Blackman reaction. The apparent effect of glucose on the rate of peroxide decomposition may perhaps be due to the greater consumption of oxygen by the glucose cells.

There is room for some disagreement as to the significance of the figures in table II, because culturing the cells with various amounts of iron results

in changing the chlorophyll content (1). Nevertheless, even cells of very low chlorophyll content show the usual characteristics of the Blackman reaction when saturated with light and carbon dioxide. The rate should then be limited by the Blackman reaction just as truly as in cells having a normal amount of chlorophyll. Yet we find an undiminished capacity to split peroxide, accompanied by a greatly reduced Blackman reaction. Neither here nor in table I is there any correlation between the rate of the Blackman reaction and the capacity to split hydrogen peroxide.

Figure 3 shows the effect of temperature on the Blackman reaction (solid curves) and peroxide decomposition (broken curves) for both species

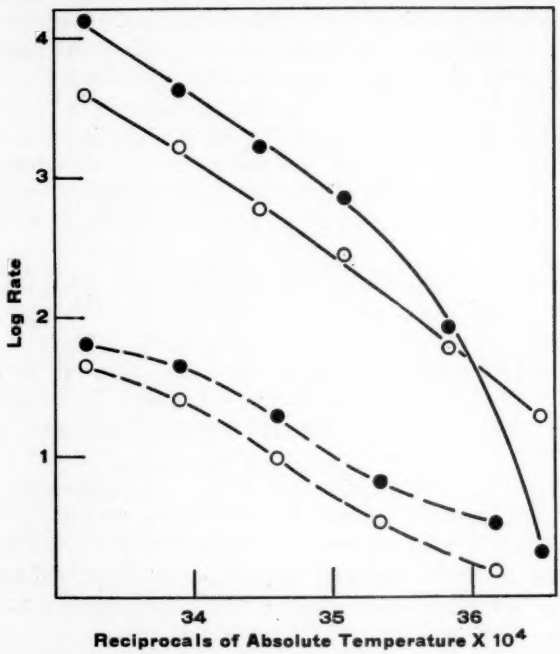

FIG. 3. Rates of the Blackman reaction and peroxide decomposition at different temperatures. Logarithms of rates are plotted against reciprocals of absolute temperatures. Solid curves are for the Blackman reaction; broken curves for peroxide decomposition. Solid circles refer to *Chlorella vulgaris;* open ones to *C. pyrenoidosa.*

of *Chlorella.* The solid circles are for *C. vulgaris,* the open circles for *C. pyrenoidosa.* The logarithms of rates are plotted against reciprocals of absolute temperatures, according to the ARRHENIUS equation. The positions of the curves on the ordinate axis are of no significance. Their slopes, which are independent of their positions, represent the temperature charac-

teristics (μ in the Arrhenius equation). The rates and temperatures are given in table III, so that a direct plot can be made if desired. Such a plot

TABLE III

RATES OF BLACKMAN REACTION AND PEROXIDE DECOMPOSITION AT VARIOUS TEMPERATURES. DATA FOR FIGURE 3

TEMPERATURE	$1/T_{Abs.}$	*CHLORELLA VULGARIS*		*CHLORELLA PYRENOIDOSA*	
		RATE	LOG$_e$ RATE	RATE	LOG$_e$ RATE
		Blackman reaction			
°C.					
1	.003650	0.5	−0.69	3.6	1.28
6	584	2.5	0.92	5.9	1.77
12	509	6.3	1.84	11.5	2.44
17	448	9.1	2.21	15.9	2.77
22	390	13.7	2.62	24.7	3.21
28	322	22.7	3.12	36.1	3.59
		Peroxide decomposition			
3.5	.003617	12.4	2.52	1.2	0.18
10	534	16.8	2.82	1.7	0.53
16	460	26.8	3.29	2.7	0.99
22	390	38.3	3.65	4.1	1.41
28	322	45.2	3.81	5.2	1.65

shows the inadvisability of comparing temperature effects from experiments at only three widely separated temperatures, as did YABUSOE, for the Blackman reaction is not, as he supposed, a linear function of temperature, but has a shape fairly characteristic for other biological processes.

The curves for the Blackman reaction are reproduced here to facilitate comparison with those for peroxide decomposition, although they are almost the same as two published earlier in another connection (**2**, fig. 6). At that time the striking difference between the two species of *Chlorella* was pointed out. While their temperature characteristics are nearly alike for the upper range of temperatures (about 10,000), they differ widely at lower temperatures, for *C. vulgaris* running to about 50,000, and for *C. pyrenoidosa* only to about 20,000.

The curves for the peroxide decomposition leave little doubt that this process is controlled by the same mechanism in both species. The upper points for *C. vulgaris* tend to fall a little low, a manifestation of the injury from M/300 peroxide mentioned in the description of methods. It might be argued that the lesser downward curvature in the case of *C. pyrenoidosa* is due to the same effect, but there is nothing in the experimental readings to confirm this.

These curves for the effect of temperature on peroxide decomposition are very like FRENCH's (**4**) figure for *C. pyrenoidosa.* He draws a straight line through his points, instead of a curve. The writers' points seem to be somewhat better fitted by a curve. The differences, insignificant in any case, may be due in part to the fact that the writers' experiments cover a range of about 25°, while FRENCH's cover a range of 19°, and in part to his use of a method rather different from the writers'.

There is a remote resemblance between the curves for Blackman reaction and peroxide decomposition for *C. pyrenoidosa,* but surely insufficient to serve as evidence for a relationship between the two processes. Between the two curves for *C. vulgaris,* there seems to be no resemblance whatever.

Conclusions

It is the opinion of the writers that an examination of the results presented in this paper suggests no significant similarity between the Blackman reaction and the peroxide decomposition. This leaves no experimental support for the hypothesis that the Blackman reaction involves the decomposition of some peroxide by catalase, but the writers wish to emphasize that neither these nor any other experiments of which they know constitute conclusive evidence that the Blackman reaction is *not* a peroxide decomposition. However, we suggest that WARBURG's "acceptor theory," abandoned in favor of the peroxide theory, is still worthy of consideration as a basis for constructing a mechanism of photosynthesis.

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TUBERIZATION OF THE COLORADO WILD POTATO AS AFFECTED BY X-RADIATION

EDNA LOUISE JOHNSON

(WITH ONE FIGURE)

Introduction

The Colorado wild potato (*Solanum jamesii*) is the only species among more than 100 different kinds of plants that the writer (**6**) has irradiated in which a plant organ has made greater growth leading to increased weight as a result of the treatment. The growth and development of the underground parts of 22 species (**7**) exposed to medium doses showed detrimental effects to adventitious, fibrous, and tap roots. Corms and bulbs of certain species of Iridaceae and Liliaceae, although less sensitive than tubers of the Jerusalem artichoke, agree in showing retarded growth. The wild potato was the only species, among those in which the underground organs especially were studied, for which a dose favoring tuber production was found.

JACOBSON (**2**) reported that by x-radiation the crop of one variety of cultivated potatoes was increased 84 per cent. in weight over the control plants, while with another variety the increase was as much as 200 per cent. He stated that not only was each tuber larger than normal but that there was an increase in the total number as well. SPRAGUE and LENZ (**9**) from preliminary experiments concluded that strong doses may reduce the number of tubers formed, but that such tubers attain a greater size so that the total production is not lowered.

In the writer's preliminary experiments (**3**) with cultivated potatoes grown in the greenhouse, tubers which were given light doses of x-rays formed more young tubers per hill than did the controls, but the average weight was less than that for the controls. Consequently the average total weight per hill was practically the same for controls and experimental plants. Since only a very limited number of cultivated potatoes could be grown in the greenhouse at one time, further experiments were carried on with the Colorado wild potato which has proved to be useful in ecological experiments (**8**) since it requires little room and matures quickly.

A summary (**1**) of the studies reporting stimulative action of x-radiation has pointed out that in the great majority of cases where it has occurred, light doses have been used. Experiments of two seasons, in which the use of light doses on the wild potato failed to give increased production, have been previously reported (**5**). Results based on 14,000 tubers obtained from the second year's experiment, indicated that the controls and experimental plants did not vary more in yield than would two groups of untreated plants.

In the study herein reported, the application of one dose of 1500 r-units to mother tubers was found to favor tuberization. Five different trials involving a large number of potatoes indicated that radiation of the unsprouted tubers resulted in only a slight increase in number of progeny and in average weight per hill, but treatment of sprouted tubers gave marked increase not only in average number and weight per hill but in average weight per tuber.

Experimentation

Unsprouted tubers treated with medium x-ray dosage

Tubers were sorted so that those which were to serve as controls were of practically the same size and weight as the lot to be treated. Immediately after irradiation with a medium dose, the experimental tubers were planted either in plots in the University greenhouse, or outside in garden soil. In all cases, the controls alternated in both directions with treated tubers. When the crop was harvested about four months after planting, care was taken to sift the soil in order that even the very small tubers should not be lost.

TABLE I

Production from unsprouted tubers irradiated with medium doses

	No. of hills	Av. no. tubers per hill	Difference in treated plants	Av. wt. per hill	Difference in treated plants	Av. wt. per tuber	Difference in treated plants
	Tubers grown in greenhouse (Set-up for dose: 51 K.V. 7½ ma. 30 cm. 25 min.)						
			%	*gm.*	%	*gm.*	%
Control	47	16.5		9.5		0.58	
Irradiated	57	17.0	+3.0*	11.1	+16.8	0.65	+12.1
	Tubers grown in garden plot at altitude of 8000 ft. (Set-up for dose: 56 K.V. 5 ma. 30 cm. 33 min.)						
Control	50	19.5		13.8		0.71	
Irradiated	51	20.3	+4.1	16.7	+21.0	0.82	+15.5
	Tubers grown in garden plot at altitude of 5600 ft.) (Dose: 1510 r-units)						
Control	48	20.3		22.6		1.1	
Irradiated	43	20.8	+2.5	20.3	−10.2	1.0	− 9.1
	Totals from controls and 3 groups of unsprouted irradiated tubers						
Control	145	18.8		15.3		0.81	
Irradiated	151	19.2	+2.1	15.6	+ 2.0	0.81	

* In tables I and II of this paper, the plus sign indicates that the treated plants exhibited greater growth than the controls; the minus sign that the treated plants showed less growth.

In all cases the average number of tubers per hill, average weight per hill, and average weight per tuber were determined. These are recorded in table I. In one group which was grown in the greenhouse, it was found that the average length of time for sprouts of the irradiated tubers to appear aboveground was 36 days, whereas the average for the controls was 43 days. Dates of sprouting in the other groups were not recorded. In some cases experimental plants blossomed earlier than the check plants. Results from the three groups reported in table I indicate that when unsprouted tubers are treated with medium doses of x-rays there is a slight increase in number of progeny and in average weight per hill.

Sprouted tubers treated with 1500 r-units

Tubers in the late spring were planted in soil in 3-inch paper pots. When the shoots had reached a height of from 1 to 2 cm., they were treated with a dose of 1500 r-units and placed in the ground outdoors without disturbance of the roots. Results obtained the first year indicated that this treatment was particularly favorable, for the number of tubers per hill was increased 59 per cent., the weight per hill 110 per cent., and the weight per tuber 30 per cent. The experiments, when repeated the following summer, confirmed the results of the previous year; 50 hills each of control and treated sprouted tubers yielded 79 and 113 tubers per hill respectively. The average weight of the progeny from the treated mother tubers was increased 50 per cent. and the experimental tubers averaged heavier than those from check plants (table II).

TABLE II

Production from sprouted tubers irradiated with 1500 r-units

	No. of hills	Av. no. tubers per hill	Difference in treated plants	Av. wt. per hill	Difference in treated plants	Av. wt. per tuber	Difference in treated plants
	Tubers grown in garden plot at altitude of 5600 ft.						
			%	*gm.*	%	*gm.*	%
Control	30	19.9		17.5		0.88	
Irradiated	34	31.8	+59.6	36.8	+110.3	1.15	+30.7
	Tubers grown in garden plot at altitude of 5600 ft.						
Control	50	79.2		53.6		0.67	
Irradiated	50	113.4	+43.1	80.5	+ 50.2	0.71	+ 6.0
	Totals showing production from controls and 2 groups of sprouted irradiated tubers						
Control	80	57.0		40.1		0.70	
Irradiated	84	80.4	+41.0	62.8	+ 56.6	0.78	+11.4

Figure 1 represents the entire crop produced the second year. The small size of the tubers can be appreciated when it is known that 3961 prog-

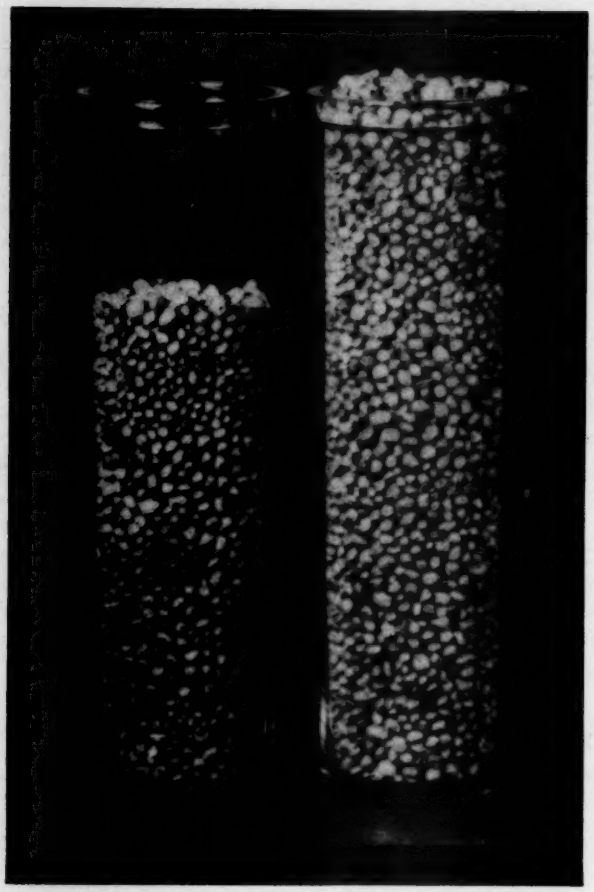

FIG. 1. Colorado wild potato tubers. Left, 3961 tubers from 50 control mothers; right, 5668 tubers from 50 sprouted mother tubers treated with 1500 r-units. Average weight of tubers is approximately 0.7 grams.

eny from 50 control mother tubers are in the jar on the left and the 5668 offspring produced by the 50 irradiated sprouted mother tubers are on the right. The average weight of the tubers is about 0.7 grams.

The application of the correct dose of x-rays favors the production of wild potato tubers. Radiated unsprouted tubers produced a crop with a slight increase in number of tubers and also in average weight per hill. Treated sprouted tubers gave striking increases in number and weight per hill and in weight per tuber. The conclusion drawn is that the probable

increased rhizome development resulting in greater tuber production is similar to the increased aerial branching which occurs in some other members of this family (**4**) when very young plants are treated with medium doses.

Summary

1. Radiation of wild potato tubers with light doses of x-rays failed to give increased number of progeny. Treatment of unsprouted tubers with moderate doses resulted in only a slight increase in number of progeny and in average weight per hill.

2. Progeny from sprouted mothers which had been treated with 1500 r-units gave the following percentage of increase over the controls: average number of tubers per hill, 41 per cent.; average weight per hill, 56 per cent.; average weight per tuber, 11 per cent. The explanation advanced for the greatly increased production from treated tubers is that there is a probable increased rhizome development which results in greater tuber production. This is similar to the increased aerial branching which occurs in some other members of Solanaceae when young plants are treated with medium doses.

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CALCIUM AND BORON CONTENTS OF THE APPLE FRUIT AS RELATED TO THE INCIDENCE OF BLOTCHY CORK

W. A. DeLong

Introduction

In the autumn of 1935 fruit and leaf samples of variety Stark apples were collected for the purpose of studying the contents of calcium and boron as related to the occurrence of the disorder known as blotchy cork, locally called "bitter-pit." These samples were obtained mainly from orchards which are under observation in connection with the investigation of the physiological disorders of the apple now in progress in the Annapolis Valley, Nova Scotia, fruit district. The method of collection followed was to obtain from each orchard two samples of fruit. One sample consisted of apples visibly affected with blotchy cork, the other of apples showing no visible symptoms of the disorder externally, and gathered from trees which apparently did not carry any affected fruit at the time the samples were collected. The samples were obtained on September 17 and 18, some weeks before the picking of this variety became general commercially. At the time that the fruit was picked two leaves also were taken from each spur from which apples were gathered. These leaves were all combined into two composite samples. One sample was from spurs bearing affected fruit, the other from spurs bearing apparently unaffected fruit on apparently unaffected trees. In connection with the sampling process it should be noted that the tree distribution of affected fruit was very irregular, as is frequently the case with this disorder, and that sampling distribution was correspondingly restricted. Thus, samples 7, 13, and 15 were collected mainly from the top sections of the trees from which they were picked, and more or less uniform distribution on the tree of affected fruit was found only in the case of samples 1 and 17. The general information concerning these samples is given in table I, in which the odd-numbered samples represent affected fruit (or leaves), and the even-numbered samples apparently unaffected fruit (or leaves).

Experimentation

The fruit samples were prepared for analysis in the usual manner by paring and drying, analysis being made on the pulp only. The leaf samples were simply dried. Determinations of calcium were made according to the procedure recommended for the analysis of the ash of fruit products by the Association of Official Agricultural Chemists. For the boron determinations 40-gm. samples of the dried apple pulp were taken. To each sample was added an excess of 10 per cent. potassium carbonate solution, together with sufficient water thoroughly to moisten the apple tissue. The mixture was

TABLE I

FRESH WEIGHT OF AFFECTED AND UNAFFECTED APPLES

SAMPLE NO.	TYPE OF ORCHARD SOIL	NO. OF FRUIT IN SAMPLE	AV. FRESH WEIGHT PER APPLE
			gm.
1	Sand, with sand subsoil	30	130
2	Sand, with sand subsoil	30	114
3	Loam, with clay subsoil	30	126
4	Loam, with clay subsoil	30	140
5	Loam, with clay subsoil	30	135
6	Loam, with clay subsoil	30	107
7	Sandy loam	30	163
8	Sandy loam	30	124
9	Sandy loam	30	116
10	Sandy loam	30	118
11	Sandy loam	28	115
12	Sandy loam	28	119
13	Sandy loam	30	108
14	Sandy loam	30	104
15	Sandy loam, with sand subsoil	30	117
16	Sandy loam, with sand subsoil	30	106
17	Clay loam underlain by sandy loam	30	161
18	Clay loam underlain by sandy loam	30	158
19	Composite leaf sample	.....	.....
20	Composite leaf sample	.....	.....
X	Sandy loam (fruit of 1935 crop)	38	100
	Sandy loam (fruit of 1934 crop)	40	120

then evaporated to dryness and ignited. The carbonaceous residue so obtained was made acid, lixiviated, again made alkaline with potassium carbonate solution, and the process repeated until ashing was complete. Ignition was at a temperature of about 450° C. The combined filtrates were made alkaline with potassium carbonate solution, evaporated to a small volume, transferred to 50-ml. volumetric flasks, and made up to the mark. Suitable aliquots were taken from the solutions so prepared for the colorimetric estimation of the boron content by the method of SMITH (**4**). This procedure has been found to be readily applicable to the determination of the amounts of boron found in apple tissue; the other ash elements present and the amounts of potassium chloride added during the ashing process exerted no measurable effect on the color change of the quinalizarin indicator. The method is convenient and rapid, and no difficulty has been experienced in obtaining duplicate determinations agreeing within the limits of accuracy of the procedure. Further, the results obtained were found, in the case of six solutions tested, namely, those from samples 1, 2, 3, 4, 5,

and 7, to be in agreement in respect to relative order of boron content with quantitative spectroscopic analyses for this element (3).

TABLE II

ANALYSIS OF SAMPLES OF AFFECTED AND UNAFFECTED APPLES

SAMPLE NO.	PERCENTAGE ASH	CALCIUM IN P.P.M. OF FRESH WEIGHT	BORON IN P.P.M. OF DRY WEIGHT
	%	*p.p.m.*	*p.p.m.*
1	0.234	36	11
2	0.320	36	7
3	0.239	32	16
4	0.256	54	19
5	0.289	36	22
6	0.245	54	16
7	0.329	33	11
8	0.231	46	7
9	0.261	32	11
10	0.236	51	11
11	0.252	28	19
12	0.249	38	22
13	0.293	30	7
14	0.240	55	7
15	0.261	36	16
16	0.233	51	14
17	0.296	28	11
18	0.271	44	22
19	.....	.....	27
20	.....	.....	38
X	0.287	44	22 (1935 crop)
X	0.235	50	19 (1934 crop)

Discussion

Examination of the results of the analysis (table II) fails to reveal any direct relation between the boron and calcium contents of the fruit, although, in view of the prevailing opinion that boron influences the calcium metabolism of the plant, some such relation might have been expected. Nor does it appear that there is any consistent relation of boron content to the incidence of blotchy cork. Yet, this too might have been expected to occur in view of the amelioration in the symptoms of other cork disorders, such as internal cork (corky core), obtained by boron treatment (1). In the case of the calcium content, however, the relation already reported (2), that a low proportion of calcium per unit of fresh weight of tissue is associated with the occurrence of blotchy cork, is again confirmed. This relation is quite marked in all cases examined in the present study except that of samples 1 and 2, in which the apparently unaffected fruits of sample 2 have essentially the same

calcium content as the affected fruits of sample 1. However, the recorded history of this orchard shows that practically all trees in it may be regarded as susceptible to blotchy cork. Storage results on fruit collected in 1935 confirm this opinion. Further, both samples 1 and 2 show a relatively low calcium content. It is therefore considered that this apparent exception to the rule, that fruits affected with blotchy cork are low in calcium, is actually more apparent than real.

Summary

1. Parallel samples of variety Stark apples, some of which were visibly affected with blotchy cork and others which were apparently unaffected, were collected from a number of orchards and analyzed for calcium and boron content.

2. The results obtained do not support the hypothesis that blotchy cork is a result of boron deficiency, nor do they indicate that the boron and calcium contents of the fruit of the apple are closely related.

3. The results confirm the previous finding that blotchy cork is associated with a relatively low calcium content of the fruit.

The writer wishes to express his sincere appreciation for the cooperation of all those who have assisted in making this work possible. He extends thanks especially to Mr. C. A. Horton of the Macdonald Physics Laboratory, McGill University, who made the spectroscopic boron determinations, and to Professors L. C. Harlow and A. D. Pickett, and their colleagues of the Nova Scotia Department of Agriculture who assisted in many ways.

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BRIEF PAPERS

METHOD OF STIRRING GASES WITHIN A CLOSED CHAMBER

(WITH ONE FIGURE)

In various types of investigations it is often desirable to circulate gases within a tight chamber by a simple method which allows for ready adjustment of the rate of circulation. This is especially true in measuring respiration and carbon fixation by plants or respiration by animals. A suitable apparatus has been devised for this purpose. In principle the method consists of rotating a fan inside of a chamber by means of a magnetic coupling which operates through the glass wall of the chamber in such a way as to cause the inclosed fan to turn synchronously with a revolving electromagnet on the outside of the chamber.

A convenient arrangement of such a device is shown in figure 1. A

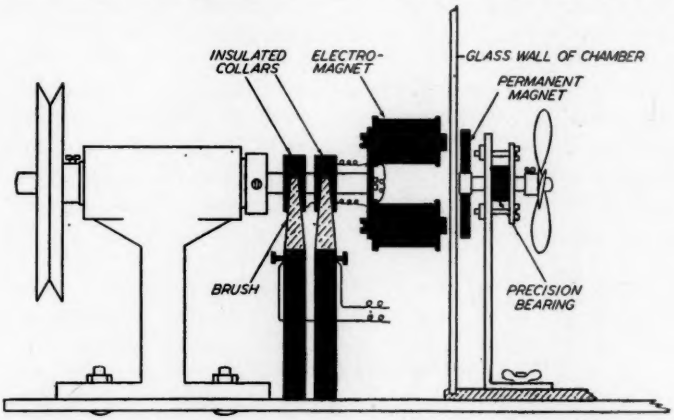

FIG. 1. Apparatus for stirring air within a closed chamber.

shaft, bearing an electromagnet and two insulated metal collars on one end, is supported by means of a suitable bearing. Copper brushes rest against the surface of the collars and carry electricity to the circuit of the electromagnet. A pulley is attached to the opposite end of the shaft and connected with a belt to a small motor by means of which the electromagnet is rotated. This part of the apparatus is mounted outside the chamber so that the ends of the core of the electromagnet are about one-sixteenth of an inch from the glass wall of the chamber and rotate in a plane parallel to it.

The removable fan unit that operates inside of the chamber consists of an L-shaped brass strip to which a precision bearing is attached by means

of a housing. The bearing supports a brass shaft, on one end of which is attached a permanent magnet, and on the other end, a fan. The unit is clamped in place by means of a bolt in the floor of the chamber so that the permanent magnet is about one-sixteenth of an inch distant from the wall of the chamber.

When the energized electromagnet rotates, the permanent magnet revolves synchronously with it, and in a plane parallel to the wall of the chamber. A suitable method of starting the apparatus is to cause the belt to slip and then gradually tighten until the maximum speed is reached as the two magnets remain synchronized only when subjected to a gradual rate of acceleration. Four units, equipped with four inch fans and electromagnets similar to those used in standard telegraph sounders, can be operated by current from an automobile generator. The amount of gas agitated by a given fan is varied by changing the speed at which the fan revolves and this is readily accomplished through the use of different sized pulleys.—JOHN W. MITCHELL and WILLIAM E. MARTIN, *University of Chicago.*

NOTES

Denver and Indianapolis Meetings.—The American Society of Plant Physiologists has decided to meet with the A. A. A. S. at Denver in June, 1937, and at Indianapolis in December, 1937. Dr. H. F. CLEMENTS, Washington State College, is chairman of the program committee for the Denver meeting, and Prof. R. B. WITHROW, Purdue University, is chairman of the program committee for the Indianapolis meeting. The program committee has been authorized to determine the number of papers which may be accommodated at any given session, and to refuse papers which come in after the maximum number have been accepted for a given meeting. In addition, the committee may set time limits for all papers, and at future meetings each author is limited to one paper on the society's program. The restrictive measures are necessary, and will be administered in the interests of the entire membership. Sympathetic cooperation with the committees will solve any difficult situations that may arise.

The date of the Denver meeting has been fixed for the week of June 21–26, 1937. The programs of the plant physiologists will be held on June 22–25 of this week. A symposium on hormones is being arranged by Dr. F. W. WENT, and other symposia are contemplated. Inquiries regarding details of the meeting may be sent to the secretary, Dr. W. F. LOEHWING, or to the chairman of the program committee. General headquarters of the A. A. A. S. will be the Cosmopolitan Hotel. Other assignments have not yet been made.

Western Section.—The election held by the Western Section has resulted in the choice of Dr. J. P. BENNETT, University of California, as chairman; Dr. W. M. ATWOOD, Oregon State College, as vice-chairman; and Dr. H. F. CLEMENTS, State College of Washington, as secretary. The second annual meeting of the section coincides in time and place with the summer meeting of the society in Denver. It is hoped that many members residing outside of the regional territory covered by the Western Section may go to Denver, for it is an attractive meeting place, and the meeting offers opportunity for mid-western and far-western physiologists to become better acquainted. Fine fellowship, and the inspiration of scenic grandeur, will be enjoyed by all who come.

New England Section.—The annual meeting of the New England Section of the American Society of Plant Physiologists will be held under the auspices of the Rhode Island State College, at Kingston, Rhode Island, on May 14–15, 1937. Those who are planning to attend the meeting, or to take part in its discussions and deliberations are requested to inform Dr. B. E.

GILBERT, at Kingston, so that ample provision may be made for the comfort and convenience of all visiting scientists. A unique feature of the program will be sessions devoted to the problems of teaching plant physiology in different types of institutions.

Frederick Frost Blackman.—In celebration of the seventieth anniversary of his birth, this number of PLANT PHYSIOLOGY is dedicated by the American Society of Plant Physiologists to FREDERICK FROST BLACKMAN, who for many years was reader in plant physiology at the University of Cambridge. Dr. BLACKMAN was born in London, July 25, 1866. His parents were FREDERICK BLACKMAN, a doctor in South London, and CATHERINE (FROST) BLACKMAN, daughter of WALTER FROST, a doctor in Dorset. In 1917 he married ELSIE CHICK, daughter of S. CHICK, J. P. They have one child, a son.

Professor BLACKMAN's early education was obtained at the Mill Hill School, London, which he attended from 1878 to 1883. During several years immediately succeeding this period at the Mill Hill School, he prepared for the medical profession, and was an entrance scholar at St. Bartholomew's Hospital, London, in 1883. In 1886 he was awarded a gold medal by the University of London for his work in organic chemistry. Having become now deeply interested in the natural sciences, he gave up his medical studies in 1887, and entered St. John's College, Cambridge. Here he studied botany and plant physiology, and in 1891 he was appointed University Demonstrator in Botany. He was elected a fellow of St. John's College in 1895, and in the following year received the degree of D.Sc. (plant physiology) from London University. He was appointed University Lecturer in Botany (Musci and Algae) in 1897, and finally Reader in Botany at Cambridge in 1904, as successor to Sir FRANCIS DARWIN. This position he has filled with great distinction for 32 years. Many honors came to him in recognition of his leadership in his field. In 1906 he was elected Fellow of the Royal Society of London, and in 1908, president of the Botanical Section of the British Association for the Advancement of Science. From 1914 to 1917 he was a member of the council of the Royal Society of London, and in 1921 was awarded the Royal Medal of the Royal Society. Two years later he was Croonian Lecturer of this organization in recognition of his work on plant respiration. Honors also came from foreign countries as his fame spread to all nations. In 1926 he was elected a foreign member of the Academy of Science, Amsterdam, and in 1935 he was elected a CHARLES REID BARNES life member of the American Society of Plant Physiologists. In 1930 he served as president of the Physiology Section of the 5th International Botanical Congress which was held at Cambridge. His retirement as Emeritus Reader

in Plant Physiology, University of Cambridge, in 1936, permits leisure to continue his fruitful investigations on plant respiration.

It is appropriate to record a few of the many contributions which Professor BLACKMAN has made to science. Following his early work on organic chemistry with Dr. S. RUHEMANN, he began publishing in 1895 a series of experimental researches on vegetable assimilation and respiration. The first papers dealt with the paths of gaseous exchange in leaves, was followed by work on temperature and photosynthesis, optima and limiting factors, and a long list of papers contributing to our knowledge of photosynthesis and respiration. In 1928 he and his students started a series of papers which are essentially analytic studies in plant respiration. As emeritus reader, Professor BLACKMAN continues his work in this field.

Dr. BLACKMAN is versatile in his interests, and is fond of the fine arts, music, archeology, and is an amateur architect. He loves walking as a recreation, and is fond of travel. For many years he served as syndic of the Fitzwilliam Museum, Cambridge; was vicepresident of the University Musical Society; life member of the musical club; a member of the council of St. John's; a member of the Government Food Investigation Board; and director of the Cambridge Instrument Co. He is a life Fellow of St. John's College, member of the Athenaeum Club, and of the Wine and Food Society, London.

In dedicating this number of PLANT PHYSIOLOGY to Professor BLACKMAN, the American Society of Plant Physiologists recognizes the great value and significance of his contributions, and rejoices with him in celebration of the seventieth anniversary of his birth, which was reached on July 25, 1936. It is intended by this dedication to convey to Dr. BLACKMAN the hearty congratulations of American plant physiologists, and their cordial good wishes for a long and happy continuation of his distinguished service to humanity.

James Bertram Overton.—It is with keen regret that we must record the death, on March 18, 1937, of Dr. JAMES B. OVERTON, Professor of Plant Physiology at the University of Wisconsin. Dr. OVERTON was born at Richmond, Michigan, on December 23, 1869. He was educated in the public schools of Michigan, and received his Ph.B. degree from the University of Michigan in 1894. He began teaching at Black River Falls, Wisconsin, where he was assistant principal of the high school during 1894–95. He became senior master in mathematics at St. John's Military Academy at Delafield, Wisconsin, in 1895, a position which he held for several years. In the summer of 1900, and in 1901, he was assistant in botany at the University of Chicago, where he enjoyed association with Dr. J. M. COULTER, Dr. C. R. BARNES, and DR. C. J. CHAMBERLAIN. He received the Ph.D. degree

from Chicago in 1901, his thesis subject being "Parthenogenesis in *Thalictrum purpurascens.*" For three years, following his work in Chicago, he was Professor of Biology at Illinois College, Jacksonville, Illinois, a connection which was honored in 1930 by the conferring upon him by that institution of the honorary Sc.D. In 1903–04 he became research assistant with the Carnegie Institution of Washington, and spent some time at the University of Bonn. He began his long service at the University of Wisconsin as instructor in botany in 1904. He was promoted to an assistant professorship in 1907, became associate professor of plant physiology in 1912, and professor in 1915. In this last position he gave almost 22 years of continuous service. During the years 1925–29 he renewed his connection with the Carnegie Institution as research associate. He held membership in many state and national scientific organizations, including the American Society of Plant Physiologists. At the Boston meeting of the plant physiologists, in 1933, Dr. OVERTON was honored by election as a CHARLES REID BARNES life member of the organization in recognition of his long service and his contributions to plant physiology. The immediate cause of death was heart failure. Mrs. OVERTON and several children survive him. His death is especially regretted as marking the first break in the living link memorial to Dr. BARNES, which was initiated at the Kansas City meeting in 1925, and at the Philadelphia meeting in 1926.

Patrons.—The American Society of Plant Physiologists provides in its constitution for four classes of membership: members, life members, patrons, and corresponding members. At the Atlantic City meeting, two members who some years ago had contributed liberally to the permanent support of the society were elected as its first patrons. These two members are Dr. WALTER THOMAS, Pennsylvania State College, and Dr. C. A. SHULL, University of Chicago. Naturally, these first patrons hope that they may not be left too long as the society's only patrons. There is plenty of opportunity for others to join the group, and the permanent funds of the society cannot be oversubscribed. This is a frank invitation to many others to share in the building of financial stability of the organization.

Drawings.—Occasionally the editors receive drawings which are inconveniently large for transportation by mail or express. The page width of PLANT PHYSIOLOGY is 4.5 inches, and drawings which must be made large enough to occupy the full width of the page should be drawn to a scale that calls only for one-half or one-third reduction, that is, nine to thirteen inches in width. Extra charges are encountered when excessive or unusual reduction in size must be made. Lines and figures should always be adapted in

thickness and size to the reductions contemplated. There seems to be no excuse, except lack of understanding, perhaps, for drawings made up to occupy several feet each way. In case the original drawings are large, it is suggested that properly reduced photographs be sent for reproduction. The author could then judge accurately whether the original lines and figures were heavy enough to show properly when reduced to the size which can be used in the journal.

Vacation.—The editor-in-chief of PLANT PHYSIOLOGY is spending the spring quarter of 1937 *in absentia* from the University of Chicago. It is suggested that those who have papers in preparation for the journal might find it convenient to hold them until about June 10, at which time the office will again be open. No attempts will be made to reply to first class mail during this period.

Chromatographic Adsorption Method.—About thirty years ago TSWETT published the first account of his chromatographic method of isolating carotenoid pigments from solutions. The usefulness of the method has been recognized, and extended, until now it is of major importance in the problems of pigment separation. A monograph devoted to the chromatographic technique, *Die chromatographische Adsorptionsmethode,* has been published by the Vienna press of Julius Springer. The authors are Dr. L. ZECHMEISTER and Dr. L. v. CHOLNOKY.

The work is presented in two main sections. The general section discusses the field in which the method is useful, its history, theoretical background, the relations between chromatographic behavior and constitutional formulae, and the methods applied to various pigments and colorless substances.

The special section discusses the application of the method to natural coloring matters, to artificial pigments, and to colorless and weakly colored substances. These special discussions will prove most helpful to those engaged in pigment study. The rapid extension of our knowledge of the carotenoids has been greatly favored by the development of efficient means of separation of closely related chemical compounds. The chromatographic method makes possible identification of the different pigments, and facilitates their quantitative estimation.

The book is published in brochure form, contains 191 pages of text, 45 figures, a bibliography containing several hundred citations, author and subject indices; the total number of pages is 231. The price of this excellent monograph is 14.40 RM per copy. Address orders to Julius Springer, I Schottengasse 4, Wien.

Contractile Cells of Plants.—Volume X of the *Protoplasma Monographien,* Gebrüder Borntraeger, Berlin, is entitled *Die kontraktile Zelle der Pflanzen.* The author is SILVIA COLLA, Privatdozent in Botany, University of Turin. Following a brief introduction there are seven chapters, dealing with material and methods, morphology of contractile cells, mechanism of movement of various contractile organs, the various phases of contractile phenomena, the laws governing the contraction and movements of isolated cells, physico-chemical changes during contraction, and a comparison of plant contractions with other cellular contractions, such as striped muscle tissue, contractile cells and organs among the protozoa, and other movements in plants. There is a long literature list (333 titles), and brief author and subject indices. There are 77 illustrations and 168 pages.

The monograph brings together much interesting and valuable information concerning the power of protoplasm to contract, and to move various organs. Especially valuable are the summaries of what is known of the physico-chemical changes occurring in cells during contraction, such as changes in potential, pH, rH, concentration of crystalloids, colloidal changes, and modifications of permeability. This volume should receive a hearty welcome from physiologists and biologists generally, because of its elucidation of the problems of protoplasmic behavior. The price, in the usual red cloth binding, is RM 12. Orders for the book should be addressed to the publishers, Gebrüder Borntraegar, Berlin W 35, Koester Ufer 17, Germany.